VOLUME 29 JUNE 1951 NUMBER 3

Canadian Journal of Medical Sciences

Editor: J. B. COLLIP

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF MEDICAL SCIENCES

This was formerly *Section E, Canadian Journal of Research*. The change to the new name took place January 1, 1951. The CANADIAN JOURNAL OF MEDICAL SCIENCES is published six times annually.

The CANADIAN JOURNAL OF MEDICAL SCIENCES is published by the National Research Council of Canada under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. Matters of general policy are the responsibility of a joint Editorial Board consisting of members of the National Research Council of Canada and the Royal Society of Canada.

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CORRECTION

On p. 77 of the April issue of this Journal (Canadian Journal of Medical Sciences, Volume 29, Number 2) please insert the following acknowledgment. It should appear above the heading **References.**

Acknowledgment

Fig. 1 has been reproduced by courtesy of The Journal of Biological Chemistry and Figs. 2–6 by courtesy of The Archives of Biochemistry.

Canadian
Journal of Medical Sciences

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 29 JUNE, 1951 NUMBER 3

EFFECT OF HIGH DOSES OF AMYTAL ON SKELETAL MUSCLE[1]

BY BERNARD E. RIEDEL AND MERVYN J. HUSTON

Abstract

Sodium amytal, 350 mgm. per kgm., injected intraperitoneally into rats caused a marked depression in the response of normal and denervated striated muscle to electrical stimulus. It is believed that the effect is due primarily to a direct action on the muscle. Sodium amytal and curare have a synergistic effect in depression of muscle fibers.

Introduction

In a previous communication (6) it was shown that sodium amytal, 190 mgm. per kgm., injected intraperitoneally into rats caused a transient increase in response of normal and denervated striated muscle to electrical stimulus. No significant depression was noted at that dose level although certain somatic neuromyal phenomena have been reported to be depressed by barbiturates (2, 3, 8). It was thought of interest to test the effect of very high doses of sodium amytal on the somatic system of the rat.

Method

The experimental method was the same as that previously described (6). Rats were used whose spinal cords had been sectioned the preceding day. The animals were fixed on a small table and the response of the gastrocnemius muscle to stimuli at 10-sec. intervals from an electronic stimulator was recorded by an isometric spring on a revolving drum.

Since the dosages were beyond the lethal dose it was necessary to apply artificial respiration. This was done by passing compressed air through a revolving stopcock into a tracheal cannula (7).

Results

Ten rats were injected intraperitoneally with 350 mgm. per kgm. of sodium amytal. In all experiments the response of the gastrocnemius muscle was markedly decreased, in two cases to zero. The mean decrease was to 16%.

[1] *Manuscript received in original form March 20, 1950, and as revised November 20, 1950. Contribution from the School of Pharmacy, University of Alberta, Edmonton, Alta.*

All of the animals, with the exception of one whose depression had been to zero, showed some degree of recovery. The mean of the recovery was up to 44% which was an increase of 28% above the level of maximum depression.

Table I compares the mean of the experimental results with the mean of 30 control animals. It is apparent that the results are significant.

TABLE I

EFFECT OF SODIUM AMYTAL ON THE RESPONSE OF THE GASTROCNEMIUS MUSCLE OF THE RAT

Figures represent percentage of initial contraction tension. The number of animals used in each case was 10 experimental and 30 control

Dose, mgm. per kgm.	State of muscle	Mean max. rec.	Time, min.	Mean control at that time	Difference	Mean max. rec.	Time, min.	Mean control at that time	Difference
350	Normal	16	111	90	74	44	352	86	42
350	Denervated	10	128	82	72	12	348	77	65
350	Curarized*	7	284	76	69	7	489	67	60
190	Curarized*	32	206	78	46	72	446	67	5

* *Intocostrin three units per kgm.*

Half the animals showed a brief preliminary increase of response but the mean increase was not significantly raised. It would appear that the depressant effect of this dose of the drug obscures and counteracts most of the stimulant action seen at 190 mgm. per kgm. (6).

Denervation Experiments

In an attempt to locate the site of the depressant action a series of experiments was carried out using denervated muscles. The animals were prepared by aseptic section of the sciatic nerve in one thigh. After a period of five days to allow for nerve degeneration these limbs were studied in the same manner.

In 10 experiments all the legs showed a marked decrease of response, six of them to zero. Only four showed any recovery and the mean recovery was much less than that of normal muscle.

Eight of the 10 experiments showed a transient preliminary increase with a mean of 102.3%.

When the effect on denervated muscle, relative to its control, is compared with the effect on normal muscle (Table I) it is apparent that the depressant action is essentially the same in both cases. Therefore the site of action would appear to be on the muscle fibers rather than the neuromyal junction.

Curarized Muscle Experiments

In two series of 10 rats each, sodium amytal, 190 mgm. per kgm. and 350 mgm. per kgm., was injected intraperitoneally into rats which had been curarized by the administration of Intocostrin (Squibb) three units per kgm.

At 350 mgm. per kgm. only two experiments showed any preliminary increased response and the mean was not raised. All the legs rapidly decreased in response, in seven cases to zero. Only two legs showed any recovery. At 190 mgm. per kgm., four of 10 rats showed some preliminary increase of response though the mean was not significantly raised. The rate of decrease was about the same as with the higher dosage but in only one experiment was the response reduced to zero. The extent of recovery was much better (See Table I).

Discussion

It was previously shown (6) that sodium amytal at a dose of 190 mgm. per kgm. produced a transient increase of response of normal (115.7%) and denervated muscle (107.8%). No depression was observed. With a dose of 350 mgm. per kgm., the mean response of normal muscle showed no increase and that of denervated muscle an increase to 102.3%. Both normal and denervated muscle showed a marked decrease. It is probable that the depressant effect prevents the manifestation of any stimulant action. The depressant action would appear to be almost entirely on the muscle, since the rate and extent of decrease of normal and denervated muscle approximate each other rather closely when comparison is made relative to their respective controls.

Huston, Martin, and Dille (3) obtained a depressant effect on perfused dog muscle with concentrations of 400 mgm. % for pentobarbital and 200 mgm. % for seconal. A dose of 350 mgm. per kgm. approaches this blood level. These workers, however, using lower concentrations demonstrated a depressant effect on the neuromyal junction. No neuromyal effect was demonstrated for amytal although the possibility of such an effect has not been eliminated. It may be that the depressant action on the muscle is so pronounced as to mask any neuromyal depression.

The pH of a solution of sodium amytal containing 350 mgm. per kgm. is 9.45. The pH of a solution of sodium hydroxide containing an equivalent weight of sodium to that of the amytal solution is 11.14. It was shown in our previous work (6) that the injection of such a solution of sodium hydroxide did not alter the response from that of the control curve. It is therefore believed that pH and ionic effects are not responsible for the results obtained with sodium amytal.

The increased response which sodium amytal, 190 mgm. per kgm., produces in normal and denervated muscle is not seen in curarized muscle. This might seem to indicate that the action was not directly on the muscle. However, curare, as well as blocking transmission at the neuromyal junction, has a depressant action on the muscle (4). It would seem therefore that muscle depressed by curare is not capable of responding to the stimulating effect of amytal. It is further to be noted that curare and amytal have synergistic effects in depression of muscle. Whereas sodium amytal alone, 190 mgm. per kgm., did not produce a significant depression of the muscle (6), this dose

did produce a marked decrease of response of curarized muscle as compared to the curarized controls. A similar synergism between evipal and curare has been shown by other workers (1, 5).

Acknowledgment

The sodium amytal used in this series of experiments was supplied by Eli Lilly and Company.

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A METHOD FOR ESTIMATING THE RATES OF GASTRIC SECRETION AND EMPTYING[1]

By J. A. Hildes and D. L. Dunlop

Abstract

The dilution indicator, phenol red, was used to measure rates of gastric secretion and emptying. A test meal containing 2.5 mgm. phenol red was introduced into the stomach through a tube, the stomach contents mixed, and a sample aspirated. The volume of stomach contents was calculated from the immediate dilution of the phenol red. At the end of a 20 min. test period the stomach volume was again estimated by sampling before and after the addition of 1.0 mgm. phenol red. From the known volume of gastric contents and the known concentration of phenol red the rates of gastric secretion and emptying were calculated from the equations

$$(1)\ \log \frac{Q}{Q_0} = \frac{B}{B\text{-}A} \log \frac{V}{V_0},$$

$$(2)\ V = V_0 + (A\text{-}B)t\ .$$

Q_0 and Q are the quantities of dye at the beginning and end of a test period of t minutes' duration; V_0 and V are volumes of stomach contents at these times and A and B are the rates of secretion and emptying respectively. The results of 10 experiments are presented.

Introduction

A variety of methods, using phenol red as a dilution indicator, have been used to measure the rate of gastric secretion (1, 2, 4, 5, 9, 11). That proposed by Schoen and Knoefel (8) seems to be theoretically the most sound. The principle of the dye dilution method is that a decrease in concentration of the indicator dye is effected by gastric secretion whereas a decrease in quantity of dye present in the stomach is effected by emptying through the pylorus. To apply the principle, therefore, it is required to know not only the concentration of phenol red at the beginning and end of a test period, but also the volume of stomach contents at these times. By taking into account the fact that gastric secretion and emptying are occurring simultaneously, Schoen and Knoefel (8) present formulae for calculating secretion and emptying rates. This has been applied to the serial estimation of gastric secretion rates under various circumstances (7, 3).

An objection to this method is that the gastric volume is obtained by measuring the aspirated contents, and in the present paper a modification to overcome this objection is proposed. The volume in the stomach was determined by the immediate dilution of a known added quantity of phenol red, rather than by aspirating the stomach contents.

[1] *Manuscript received January 13, 1951.*

Contribution from the Department of Physiology and Medical Research, The University of Manitoba, Winnipeg, Man.

Material and Methods

Subjects

Ten experiments were carried out on seven healthy male medical students.

Experimental Procedure

After the subject had fasted overnight, a Levin tube was passed into his stomach and oral suction was applied to prevent the swallowing of saliva. The subject reclined on his left side. A sample of fasting juice was aspirated and the meal, consisting of 250 ml. of 12% glucose and 2.5 mgm. of phenol red, was introduced through the tube. The gastric contents were immediately mixed by attaching a 50 ml. syringe to the tube and moving the plunger back and forth 10 times as quickly as possible. A sample was then aspirated for phenol red determination. At the end of a 15-20 min. test period the stomach contents were again mixed and sampled. Immediately 1 mgm. of phenol red in 10 ml. water was added to the stomach, the contents again thoroughly mixed and a sample aspirated. Each experiment consisted of two or more such test periods.

The protocol of one of the experiments is shown in Table I.

TABLE I

PROTOCOL OF EXPERIMENT No. 6

Time, min.	Phenol red injected, mgm.	Sample No.	Volume of sample, cc.	Conc. of phenol red, mgm. %
0	2.5 with test meal			
2		1	25	0.85
22		2	25	0.79
23	1.0			
24		3	25	1.20
42		4	25	0.99
43	1.0			
44		5	25	1.69
62		6	25	1.35
63	1.0			
64		7	25	2.74
82		8	20	1.93
83	1.0			
84		9	18	4.36

Phenol Red Estimation

The procedure used was a modification of the method of Shay *et al.* (10). To 5 ml. of filtered gastric contents sufficient powdered calcium oxide was added to give a distinct pink color; 1 ml. of 0.5*N* sodium hydroxide, 1 ml. of 0.3*N* $Zn\,SO_4 \cdot 7H_2O$ and 3 ml. of 0.5*N* $Na_3\,PO_4 \cdot 12H_2O$ were added in succession. The tubes were shaken, allowed to stand 10 min., and centrifuged.

The supernatant was read in a Klett colorimeter with a 540 filter. The standard deviation of 18 estimations on gastric juice containing 1 mgm. per 100 ml. was ± 0.008 mgm. per 100 ml.

Calculation of Volume of Stomach Contents

The volume of stomach contents was calculated from the dilution of the added phenol red. The volume 22 min. after the test meal in the experiment shown in Table I is calculated as an example.

If the volume of stomach contents be x ml. and the concentration is 0.0079 mgm. per ml., then the quantity of phenol red remaining in the stomach after Sample 2 is $(x - 25)$ (0.0079) mgm. After the addition of 1.0 mgm. phenol red in 10 ml. water the concentration in Sample 3 is 0.012 mgm. per ml. Therefore $(x - 25)$ (0.0079) + 1.0 = $(x - 15)$ (0.012), which can be solved for x.

Calculation of Rate of Gastric Secretion and Emptying

In a test period of t minutes' duration let C_0 and C be concentrations of dye at times 0 and t (mgm. per ml.), Q_0 and Q be quantities of dye at times 0 and t (mgm.), V_0 and V be volumes of stomach contents at times 0 and t (ml.), and A be the rate of gastric secretion and B the rate of gastric emptying (ml. per min.).

$$\text{Then } C = \frac{Q}{V} \quad \ldots\ldots (1)$$

$$\text{and } V = V_0 + (A\text{-}B)t \quad \ldots\ldots (2)$$

The amount of dye leaving the stomach in a short time dt can be expressed as $-BCdt$

$$\text{Therefore } dQ = -BCdt \quad \ldots\ldots (3)$$

Substitute into this equation for C (Equation 1)

$$dQ = -\frac{BQdt}{V} \quad \ldots\ldots (4)$$

and substitute for V (Equation 2)

$$dQ = \frac{BQdt}{V_0 + (A\text{-}B)t} \quad \ldots\ldots (5)$$

$$\text{or } \frac{dQ}{Q} = -B\,\frac{dt}{V_0 + (A\text{-}B)t} \quad \ldots\ldots (6)$$

Integrating between the limits 0 and t

$$\log \frac{Q}{Q_0} = \frac{B}{B\text{-}A} \log \frac{V}{V_0} \quad \ldots\ldots (7)$$

Equations (2) and (7) are simultaneous equations which can be solved for A and B.

Results

The results of 10 experiments are shown in Table II. In the first test period of Experiments 5 and 8 the concentration of phenol red was found to be greater at the end of the period than at the beginning. In Experiment 3 the

TABLE II

DATA PERTAINING TO RATES OF GASTRIC SECRETION AND EMPTYING

Expt. No.	Subject	Time after test meal, min.	Stomach volume		Conc. phenol red		Quan. phenol red*		Rate of:	
			Initial, ml.	Final, ml.	Initial, mgm. %	Final, mgm. %	Initial, mgm.	Final, mgm.	Secretion, ml./min.	Emptying, ml./min.
1	D.L D.	2-22	267	240	0.86	0.84	2.29	2.00	0.4	1.7
		24-44	200	172	1.25	1.17	2.50	2.00	0.7	2.1
2	D.L.D.	1-20	241	224	0.94	0.76	2.27	1.71	2.6	3.5
		23-43	184	116	1.37	1.12	2.52	1.35	0.7	4.2
3	J.T.	1-20	265	331	0.86	0.85	2.28	2.81	0.2	−3.2
		22-40	291	172	1.12	1.06	3.27	1.83	0.7	7.3
4	E.S.	2-22	229	178	0.98	0.77	2.25	1.37	2.5	5.0
		24-43	138	147	1.39	1.15	1.92	1.69	1.4	1.0
5	G.C.	2-22	570	250	0.42	0.69	2.40	1.72	−9.6	6.4
		24-42	210	200	1.08	0.98	2.27	1.95	1.0	1.6
6	S.T.	2-22	269	239	0.85	0.79	2.29	1.88	1.0	2.5
		24-42	199	143	1.20	0.99	2.39	1.42	2.0	5.0
		44-64	103	77	1.70	1.35	1.75	1.04	1.1	2.6
		66-84	37	43	2.74	1.93	1.02	0.82	0.8	0.5
7	D.L.D.	2-22	252	198	0.90	0.87	2.27	1.72	0.4	3.1
		25-45	158	59	1.35	1.10	2.13	0.65	1.0	6.0
		48-68	19	41	2.93	1.35	0.56	0.56	1.1	0.0
8	W.C.	2-17	339	287	0.69	0.76	2.33	2.19	−2.2	1.3
		19-40	247	195	1.10	1.00	2.72	1.95	1.1	3.8
		41-60	155	95	1.50	1.28	2.33	1.21	0.8	4.0
9	S.T.	2-21	243	156	0.94	0.79	2.27	1.23	1.7	6.3
		23-41	116	93	1.45	1.08	1.69	1.01	1.7	3.0
		43-61	53	43	2.24	1.60	1.19	0.70	0.8	1.4
10	D.S.	2-20	273	210	0.84	0.73	2.29	1.53	1.9	5.4
		22-40	170	86	1.21	0.98	2.06	0.85	1.4	6.1

* *Quantity of phenol red calculated by multiplying concentration and volume.*

quantity of phenol red at the end of the first test period was greater than at the beginning. These results are attributed to inadequate mixing of the dye with the stomach contents.

In the remaining seven experiments, shown graphically in Fig. 1, rates of secretion ranged from 0.4 to 2.6 ml. per minute. The mean of 18 test periods was 1.3 ml. per minute. The rates of gastric emptying ranged from 0 to 6.3

ml. per minute with a mean of 3.3 ml. per minute. In Fig. 1 the discrepancy between the stomach volume at the end of one period and the beginning of the next is due to the volumes of the samples removed in this interval.

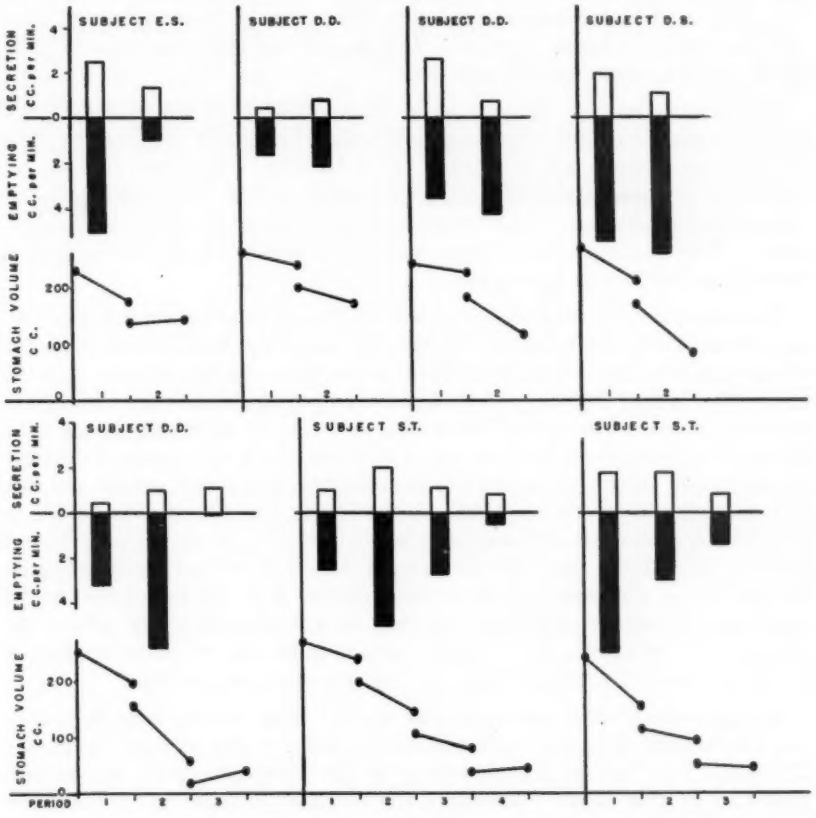

FIG. 1. Rates of secretion and emptying and the changes in volume of stomach contents in seven experiments on four subjects.

Discussion

In preliminary experiments the advantages of the dye method of volume estimation over the aspiration method was demonstrated in four subjects. In each of these an attempt was made to aspirate all the fasting juice. When no further juice could be obtained by posturing or moving the tube, 250 ml. of dye solution was injected, mixed, and a sample aspirated. The immediate dilution of the dye indicated that 40, 44, 27, and 18 ml. of juice escaped aspiration in these four subjects. This confirms that the stomach, with its mucosal folds, cannot be completely emptied by aspiration (5).

The dye method of volume estimation depends on the homogeneity of the stomach contents before each sampling. In a flask this can be achieved with one or two strokes of the syringe plunger. In the stomach, mixing is more difficult but preliminary observations showed that it was possible. Thus in one subject, after dye was introduced and the stomach contents mixed, three samples taken in rapid succession had phenol red concentrations of 0.83, 0.84, and 0.83 mgm. per 100 ml.

However in another subject, when the stomach was almost empty and when the process of obtaining samples was slow, the phenol red concentrations in three successive samples were 2.74, 2.59, and 2.41 mgm. per 100 ml. indicating progressive dilution. Thus if precise measurements of volume are to be obtained the time taken to mix and sample must be reduced to a minimum. This condition can be more readily met by the dye method of volume determination than by aspiration.

The mathematical steps in the calculation of rates of secretion and emptying are not reported by Schoen and Knoefel (8) but as the final formula is similar to our own they are presumably based on the same considerations. A major assumption is that the rates of secretion and emptying are constant throughout the test period. The results show that the rate of emptying varies from period to period and there is no reason to believe that it is constant during a single period. Hunt (6) states there is a relationship between volume and rate of emptying and this is suggested in the longer experiments illustrated in Fig. 1. Therefore the rate of emptying would be decreasing throughout the test period. In addition there will be changes in rate of emptying depending on the activity of the gastric musculature. Brooks *et al.* (3) varied the rate of emptying in their *in vitro* tests but they do not state how this affects the accuracy of the calculations. Errors arising from this would be minimized by using a meal of relatively large volume and short test periods.

A hypertonic glucose test meal was used in these experiments because it was our original intention to determine the effect of this solution on gastric function. This led, as a preliminary, to the development of the method presented in this paper. Investigations are now in hand to compare the effects of a hypertonic glucose meal with hypotonic and isotonic glucose and saline meals, with and without other gastric stimulants, such as histamine.

It is also proposed to apply similar calculations for the estimation of rates of secretion of various components of the gastric juice.

Acknowledgments

We acknowledge with pleasure our indebtedness to Prof. W. H. McEwen of the Department of Mathematics for the formula for calculating the rates of secretion and emptying, and to Dr. G. E. Delory for helpful criticism. The work was performed while J. A. H. was a Senior Medical Research Fellow of the National Research Council of Canada and D.L.D. a Scholar of the Manitoba Institute for the Advancement of Medical Education and Research.

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CIRCULATION PÉRIPHÉRIQUE ET TENEUR DES SURRÉNALES EN ADRÉNALINE ET EN ARTÉRÉNOL (NORADRÉNALINE) CHEZ LE RAT BLANC EXPOSÉ AU FROID[1]

PAR ANDRÉ DESMARAIS ET L.-PAUL DUGAL

Abstract

Experiments made on white rats exposed to cold disclosed the following facts:

(1). During the period of resistance and acclimatization to cold, the peripheral circulation shows alternate phases of vasodilation and vasoconstriction, with prevalance of the latter.

(2). Complete adaptation to cold is accompanied by the establishment of a new rate of peripheral circulation, which is a light vasodilation.

(3). Peripheral circulatory changes which occur during the period of resistance and acclimatization to cold are not accompanied with parallel fluctuations of the adrenaline and arterenol content of the adrenal medulla, but exposure to cold seems to increase gradually the adrenal content in both hormones until adaptation is reached.

Introduction

Jusqu'à ces dernières années, on croyait que la médullaire surrénale ne secrétait qu'une seule hormone: l'adrénaline. Des travaux récents ont cependant démontré la présence d'artérénol dans tous les tissus où l'on trouve de l'adrénaline (4, 5, 6, 18, 20, 21, 22, 26, 28, 32). L'artérénol est une amine primaire ne différant de l'adrénaline que par l'absence du groupe méthyl. L'artérénol serait le précurseur immédiat de l'adrénaline (1, 17, 22) au cours de la synthèse biologique de cette hormone, dont la substance mère serait la phénylalanine (1, 3, 24, 27, 33, 39).

Les études pharmacologiques effectuées avec des produits purs ont démontré que l'adrénaline agit comme vaso-dilatateur périphérique, tandis que l'artérénol est un puissant vaso-constricteur (23).

Des travaux antérieurs (10) nous avaient révélé l'existence de changements circulatoires périphériques chez le rat blanc exposé au froid. Nous avons voulu savoir, tout en complétant nos études sur la circulation, ce que devenait la teneur de la médullo-surrénale en adrénaline et en artérénol au cours des changements circulatoires observés.

Travail expérimental

A. Protocole Expérimental

Les modifications de la circulation périphérique ont été étudiées par la méthode de Chambers et Zweifach (8): voici un bref exposé de cette technique.

[1] *Manuscript received October 23, 1950.*

Contribution du Département de Recherches en Acclimatation, Faculté de Médecine, Université Laval, Québec, P.Q., avec l'aide financière du Conseil des Recherches de la Défense nationale du Canada.

On anesthésie l'animal (rat) au nembutal (50 mg./kg. par voie souscutanée) et on le place sur une planche spéciale, dans laquelle on a pratiqué une ouverture rectangulaire fermée de façon étanche par une lame de verre. C'est à travers cette fenêtre que sera fait, par transparence, l'examen microscopique des vaisseaux sanguins. Après incision de l'abdomen, au niveau du quadrant inférieur droit, on sort le mésoappendice et on l'étale sur la lame de verre. Les tissus exposés sont immédiatement recouverts de coton hydrophile imbibé d'une solution de Ringer – gélatine (1%) chauffée à 37° C. On glisse alors, entre la partie terminale de l'iléon et le caecum, un support de verre en forme de U sur lequel le mésentère se trouve étendu. On laisse alors tomber, au rythme de 60 gouttes par minute, la solution de Ringer – gélatine, chauffée à 37° C. La préparation est maintenant prête pour l'examen microscopique: on n'a qu'à glisser le microscope et le mettre au point sur le mésentère au faible grossissement (objectif: 4× – oculaire: 10×).

Après avoir repéré les vaisseaux à examiner: artérioles, ponts artérioveineux, pré-capillaires, capillaires et veinules, on fait les observations suivantes: l'application locale, sur le mésentère, d'une solution d'adrénaline suffisamment concentrée entraîne une constriction des artérioles et des précapillaires et une diminution de la circulation dans les capillaires et les veinules. En utilisant des solutions d'adrénaline de différentes concentrations, il est possible de trouver la concentration juste suffisante pour provoquer l'effet caractéristique de cette substance: c'est le seuil de réactivité des vaisseaux à l'adrénaline. Cette méthode permet de mesurer exactement la sensibilité à l'adrénaline des vaisseaux périphériques.

Pour l'extraction et le dosage de l'adrénaline et de l'artérénol, nous avons employé la méthode de Euler et Hamberg (19), qui permet le dosage différentiel de ces deux substances dans un même échantillon. Ces auteurs ont trouvé une étroite relation entre la méthode colorimétrique qu'ils emploient et les dosages biologiques. Nous-mêmes avons vérifié, par le dosage d'échantillons de titre connu, ainsi que par l'étude des pourcentages de récupération après addition de quantités connues d'adrénaline et d'artérénol à des extraits de surrénales, que l'erreur expérimentale ne dépasse généralement pas 1.0% lorsque le dosage est effectué sur des échantillons dont la concentration varie de 0.2 à 2.0 mg. %.

Nous avons effectué ces mesures sur des rats Wistars, tous mâles, recevant eau et Purina ad libitum, pesant de 140 à 160 grammes au départ, et placés dans les conditions suivantes:

Groupe 1.—animaux en état de résistance et d'acclimatation (non adaptés), à la température de 0° C.;

Groupe 2.—animaux adaptés au froid, à la température de 0° C.;

Groupe 3.—animaux maintenus à + 10° C., température à laquelle des animaux de ce poids s'adaptent facilement, car ils n'accusent qu'un léger fléchissement de la croissance dans les deux ou trois premiers jours d'exposition au froid.

Le critère employé pour l'adaptation est la croissance: les animaux en état de résistance sont ceux qui perdent du poids au froid; l'état d'acclimatation est caractérisé par le retour au poids initial; enfin, on dit que les animaux sont adaptés lorsque leur croissance se continue au delà du poids initial. Dugal et Thérien (12, 13) ont déjà démontré la valeur de ce critère dans leurs études sur la résistance et l'acclimatation au froid du rat et du cobaye.

B. Résultats

Toutes les valeurs données dans les Tableaux I et II sont des moyennes calculées d'après le nombre d'animaux mentionné; ces moyennes sont accompagnées de l'écart type calculé suivant la formule:

$$S = \sqrt{\frac{\Sigma\ (d)^2}{n(n\text{-}1)}}$$

n-1 est, en certains cas, remplacé par n-3 à cause du petit nombre d'animaux en expérience. De plus, dans le Tableau I, nous avons inclus le pourcentage de variation des valeurs aux différents points considérées par rapport à la valeur au point 0 prise comme valeur normale (100); de cette façon, il nous était possible de comparer les différents groupes entre eux.

La valeur "t", utilisée pour la définition de la probabilité, est calculée d'après la formule:

$$t = \frac{D}{S_D}$$

où D représente la différence actuelle entre la valeur normale au point 0 et la moyenne considérée, et S_D l'écart type de la différence entre ces deux moyennes.

(1). *Variations de la circulation périphérique*

Nous avons déjà décrit (10) la signification de ces mesures. Rappelons que l'hypersensibilité des vaisseaux à l'adrénaline correspond à un état de vaso-constriction avec tendance à l'ischémie, tandis qu'une diminution de sensibilité à l'adrénaline se rencontre généralement sur une préparation où la vaso-dilatation est apparente, et représente un état d'hyperémie.

Groupe 1.—les valeurs données pour ce groupe et qui apparaissent dans le Tableau I et les graphiques I et II sont les résultats de nos premières expériences sur ce sujet (10). Au cours de la résistance et de l'acclimatation au froid, il se produit une alternance de vaso-constriction et de vaso-dilatation.

Groupe 2.—l'examen du Tableau I et des graphiques I et III nous montre que, chez les animaux adaptés au froid (0° C.), la sensibilité à l'adrénaline s'est stabilisée à un niveau inférieur à la normale.

Groupe 3.—Chez ces animaux, maintenus à + 10° C., la sensibilité à l'adrénaline s'est installée, dès le premier jour, à un niveau inférieur à la normale et s'est maintenue ainsi jusqu'à la fin de l'expérience. Ces résultats apparaissent dans le Tableau I et les graphiques I et IV.

TABLEAU I

SENSIBILITÉ À L'ADRÉNALINE CHEZ LE RAT BLANC EXPOSÉ AU FROID

Groupe 1* Non adaptés, 0° C.	Jours au froid	0	1	2	4	9	10	11	14	17	18	24
	Nombre d'animaux	20	13	10	11	15	14	9	10	13	13	14
	Sensibilité à l'adrénaline	6.4	12.7	3.8	10.2	4.3	11.4	3.7	12.5	10.2	4.1	4.0
	% de variation	100.0	198.4	59.3	159.3	67.1	178.1	57.8	195.3	159.3	64.1	62.5
	Écart type (n-1)	0.09	1.43	0.54	1.15	0.37	1.03	0.37	0.95	0.99	0.35	0.26
	Valeur de "t"	—	4.40	4.72	3.30	5.64	4.85	6.92	6.35	3.84	5.89	8.37
Groupe 2 Adaptés à 0° C.	Jours au froid	0	33	43	56	64	75					
	Nombre d'animaux	8	11	9	10	8	5					
	Sensibilité à l'adrénaline	5.6	3.9	4.4	4.5	3.0	3.6					
	% de variation	100.0	68.5	78.6	80.8	53.0	63.9					
	Écart type (n-3)	0.63	0.39	0.40	0.41	0.39	0.56					
	Valeur de "t"	—	2.39	1.92	1.44	3.55	2.39					
Groupe 3 Rats maintenus à + 10° C.	Jours au froid	0	1	2	4	9	10	11	17	18	24	
	Nombre d'animaux	8	10	11	9	12	9	5	5	9	8	
	Sensibilité à l'adrénaline	6.3	3.4	3.3	3.3	3.5	5.0	3.6	2.8	4.4	3.2	
	% de variation	100.0	53.3	51.3	51.3	54.9	78.5	56.5	43.2	69.7	49.8	
	Écart type (n-3)	0.23	0.45	0.43	0.58	0.27	0.54	0.58	0.69	0.58	0.63	
	Valeur de "t"	—	5.80	6.44	4.90	8.20	2.32	4.39	4.89	3.11	4.74	

* *Ces résultats sont tirés de notre première publication sur ce sujet (10).*

TABLEAU II

TENEUR DES SURRÉNALES EN ADRÉNALINE ET EN ARTÉRÉNOL CHEZ LE RAT BLANC EXPOSÉ AU FROID*

Groupe 1	Jours au froid	1	9	10	14	17	18	24
Non adaptés, 0° C.	Nombre de paires	10	10	10	10	10	10	4
	Adrénaline	9.89	18.11	20.37	16.01	12.45	15.57	30.07
	Ecarts types	0.84	1.98	2.17	0.94	0.52	0.79	1.66
	Artérénol	4.08	6.40	8.14	8.58	8.73	6.89	15.69
	Ecarts types	0.56	0.78	1.79	1.18	0.80	0.43	1.33
	% d'artérénol	29.0	26.3	28.5	34.9	41.2	30.7	34.3
Groupe 2	Jours au froid	33	43	75				
Adaptés à 0° C.	Nombre de paires	6	8	2				
	Adrénaline	26.99	25.66	30.13				
	Ecarts types	2.11	0.83	1.41				
	Artérénol	15.47	17.46	18.74				
	Ecarts types	0.42	1.18	0.64				
	% d'artérénol	36.4	40.4	38.3				
Groupe 3	Jours au froid	4	9	10	11	18	24	
Rats gardés à + 10° C.	Nombres de paires	5	5	5	5	6	6	
	Adrénaline	6.55	10.81	13.31	12.72	17.70	16.60	
	Ecarts types	0.39	0.37	0.18	0.84	1.66	1.35	
	Artérénol	1.76	2.67	4.51	4.55	12.31	11.22	
	Ecarts types	0.09	0.17	0.19	1.48	0.22	0.56	
	% d'artérénol	21.2	19.8	25.3	26.3	41.0	40.3	

* *Les valeurs exprimant la teneur en adrénaline et en artérénol représentent la quantité totale moyenne, en micro-grammes par paire de surrénales.*

(2). *Teneur des Surrénales en Adrénaline et en Artérénol*

Les résultats sont exprimés en micro-grammes par paire de surrénales. Nous avons utilisé ce mode d'expression de préférence à la teneur en mg. par gramme de tissu, parce que l'hypertrophie de la surrénale au froid

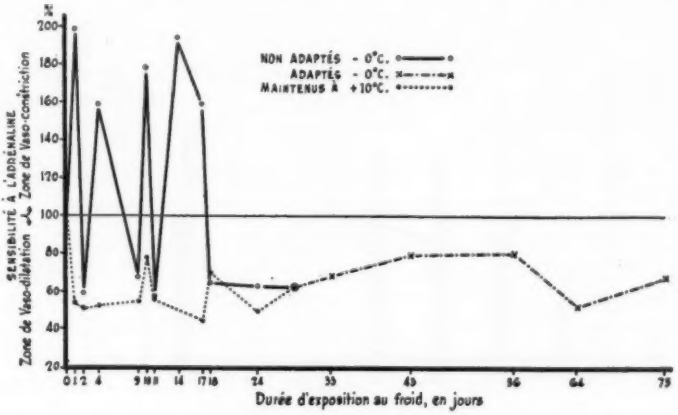

FIG. 1.

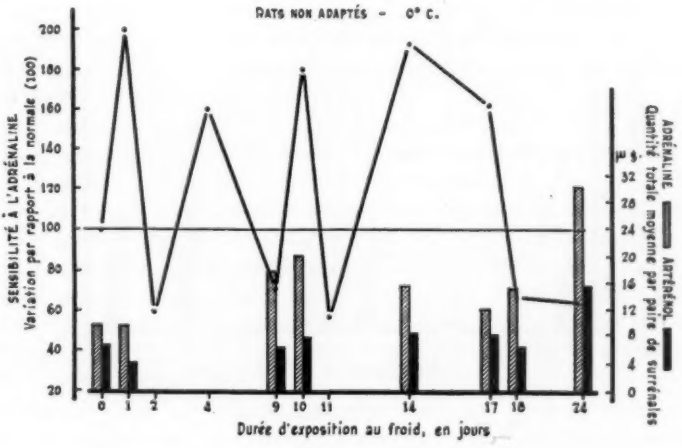
RATS NON ADAPTÉS - 0° C.
SENSIBILITÉ À L'ADRÉNALINE
Variation par rapport à la normale (100)
ADRÉNALINE
ARTÉRÉNOL
Quantité totale moyenne par paire de surrénales
Durée d'exposition au froid, en jours

FIG. 2.

n'intéresse que le cortex, à l'exclusion de la médullaire; rapporter la quantité d'adrénaline ou d'artérénol au poids des surrénales aurait donc faussé les résultats.

Les extractions et les dosages ont été faits sur des groupes de deux ou trois paires de surrénales à la fois, afin d'obtenir une plus grande précision dans

les mesures spectro-photométriques. Nous ajoutions d'ailleurs à nos extraits, à titre d'entraîneurs ("carrier"), des quantités connues d'adrénaline et d'artérénol.

Les résultats pour les trois groupes définis dans le protocole apparaissent dans le Tableau II et les graphiques II, III et IV.

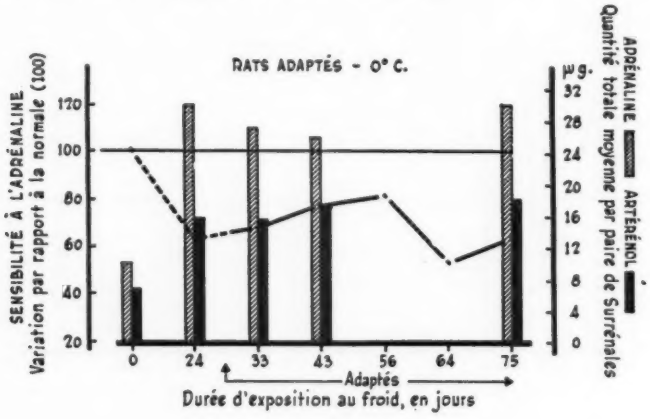

FIG. 3.

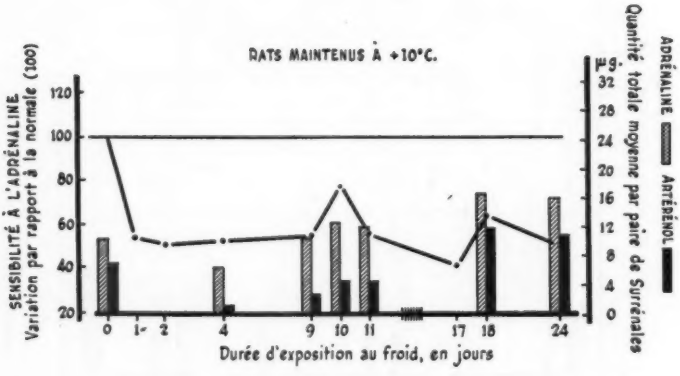

FIG. 4.

(*a*). *Adrénaline.*—La comparaison des valeurs obtenues nous montre que la teneur en adrénaline augmente au cours de l'exposition au froid, chez les animaux non adaptés (Groupe 1, Tableau II et graphique II), et davantage chez les animaux adaptés (Groupe 2, Tableau II et graphique III). La quantité d'adrénaline augmente aussi, mais moins rapidement et même après un léger fléchissement, chez les animaux soumis à la température de + 10° C. (Groupe 3, Tableau II et graphique IV).

(*b*). *Artérénol.*—La teneur en artérénol demeure à peu près inchangée au cours de la résistance et de l'acclimatation au froid (Groupe 1, Tableau II et graphique II), tandis qu'elle s'élève chez les animaux adaptés (Groupe 2, Tableau II et graphique III). Lorsque les animaux sont gardés à + 10° C. (Groupe 3, Tableau II et graphique IV), la teneur en artérénol s'abaisse considérablement au début et demeure inférieure à la normale pendant plusieurs jours, pour s'élever ensuite légèrement au-dessus de la normale.

(*c*). *Pour-cent d'artérénol.*—Nous remarquons un léger fléchissement du pourcentage d'artérénol par rapport à la teneur en adrénaline au début de l'exposition au froid, que ce soit à 0° ou à + 10° C. (Tableau II).

C. Interprétation

(1). *Variations de la Circulation Périphérique*

Chez les animaux en état de résistance et d'acclimatation au froid, nous observons des fluctuations importantes de la sensibilité à l'adrénaline. Pendant cette période, l'économie calorique est essentielle au maintien de la température corporelle. Progressivement, et les travaux sur cette question sont nombreux (2, 7, 9, 11, 14, 15, 25, 30, 38), la production calorique et le métabolisme vont s'élever au cours de l'exposition au froid; cependant, ces phénomènes d'adaptation sont lents, et l'organisme a besoin d'une protection immédiate contre les pertes de chaleur en surface. Nous constatons effectivement que pendant cette phase les valeurs de la sensibilité à l'adrénaline indiquent une augmentation de la vaso-constriction. Néanmoins, un état persistant de vaso-constriction pourrait devenir préjudiciable au bon état des tissus périphériques directement exposés au froid; c'est ce qui expliquerait les poussées de vaso-dilatation observées.

Chez les animaux adaptés à la température de 0° C., la circulation périphérique s'est établie à un régime relativement stable, différent de celui que l'on retrouve à la température normale, et représentant un état de légère vaso-dilatation. Ce phénomène semble logique, puisque, d'après les connaissances acquises sur la physiologie du froid, le métabolisme de la peau est augmenté au froid, ce qui nécessite un plus grand apport sanguin; d'autre part, il est possible que l'organisme, dans son effort d'acclimatation, dépasse les limites de l'essentiel et que, le régime métabolique étant trop élevé pour les besoins actuels, l'extra-chaleur produite soit dégagée par un accroissement de la circulation périphérique.

Ce dernier résultat sur l'institution d'un nouveau débit circulatoire chez les animaux adaptés au froid se trouve parfaitement confirmé par les observations que nous avons faites sur le groupe 3. Chez ces animaux, maintenus à + 10° C., température à laquelle le problème de l'acclimatation ne se pose pratiquement pas, nous n'avons plus ces fluctuations de la circulation périphérique observées chez les animaux non adaptés à la température de 0° C.; au contraire, à + 10° C., les animaux établissent sans délai leur régime circulatoire au même niveau que les rats qui, après 24 jours d'exposition à 0° C., avaient manifesté des signes évidents d'acclimatation.

L'établissement de ce nouveau régime circulatoire à la périphérie serait donc un des phénomènes marquant la parfaite acclimatation au froid, et serait susceptible de devenir un critère important de l'adaptation aux basses températures.

(2). *Teneur des Surrénales en Adrénaline et en Artérénol*

L'étude comparative des résultats obtenus sur la sensibilité à l'adrénaline d'une part, et sur la teneur des surrénales en adrénaline et en artérénol d'autre part, ne révèle aucune corrélation entre ces deux séries d'observations (Graphiques II, III et IV).

Nous croyons pouvoir dire, à la lumière des résultats de nos expériences, que les modifications de la circulation périphérique survenant chez nos animaux exposés au froid sont tout-à-fait indépendantes de la sécrétion médullo-surrénalienne. Selon l'opinion de certains auteurs, les sécrétions corticales (16, 34, 35, 36), certains lipides (29), ainsi que d'autres substances provenant des divers tissus de l'organisme (31, 37) seraient susceptibles de modifier la sensibilité des vaisseaux sanguins à l'action des amines vaso-motrices. C'est là, croyons-nous, qu'il faudrait chercher l'explication des phénomènes circulatoires que nous avons observés.

Quant aux changements constatés dans la teneur des surrénales en adrénaline et en artérénol, ils nous semblent difficiles à interpréter sans faire appel à des comparaisons que nos résultats actuels ne peuvent justifier.

Resumé et Conclusions

Des travaux effectués sur le rat blanc exposé au froid ont révélé les faits suivants:

(1) Au cours de la résistance et de l'acclimatation au froid, la circulation périphérique passe alternativement par des états de vaso-dilatation et de vaso-constriction, avec prédominance de ce dernier état.

(2). L'adaptation au froid est marquée par l'établissement d'un débit circulatoire périphérique différent de la normale et représentant un état de légère vaso-dilatation.

(3). Les changements circulatoires périphériques survenant au cours de l'exposition au froid sont indépendants de la sécrétion médullo-surrénalienne.

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THE INFLUENCE OF ORAL ASCORBIC ACID ADMINISTRATION ON THE ADRENAL CORTICAL RESPONSE TO ACTH[1]

By F. R. Skelton and C. Fortier[2]

Abstract

The effect of orally administered ascorbic acid (as sodium ascorbate) on the adrenal cortical response to ACTH was studied in the hypophysectomized male rat. Plasma and adrenal ascorbic acid and adrenal cholesterol levels were determined three and six hours after ACTH injection. The depletion of adrenal ascorbic acid following such treatment was partially inhibited by the administration of sodium ascorbate while the decrease in cholesterol was unaffected. These results suggest that the uptake of ascorbic acid from the increased quantity available in the plasma was sufficient to partially overcome the depleting effect of ACTH.

Introduction

Recently Dugal and Thérien (2) have reported that the adrenal hypertrophy normally produced by chronic exposure to cold was completely prevented by the administration of ascorbic acid. Further evidence of the conditioning of adrenal cortical function has been presented by Constantinides, Fortier, and Skelton (1) who showed that glucose administration significantly potentiated the adrenal cholesterol depletion produced by ACTH in the hypophysectomized rat. These observations as well as the similarity between the chemical structure of glucose and ascorbic acid have suggested a study of the effect of this vitamin on the adrenal cortical response to ACTH.

Materials and Methods

For this purpose 56 male, piebald rats averaging 150 gm. in weight were hypophysectomized and equally divided into seven groups which were then treated in the following manner. Group I was untreated and acted as the normal control. Groups II and III received a single subcutaneous injection of 2.0 mgm. of Armour's ACTH (Lot No. 60-61) in 0.2 ml. of 0.9% sodium chloride. Groups IV and V were similarly injected with saline (sham ACTH injection) and in addition received ascorbic acid (as sodium ascorbate) by stomach tube two hours later in a dose of 200 mgm. per 100 gm. of body weight. Groups VI and VII received both the ACTH and ascorbic acid treatment. One group of each pair was killed at three hours and the other at six hours postinjection so that all events were studied as a function of time. The time schedule was arranged in such a way that all animals were killed

[1] *Manuscript received February 1, 1951.*

Contribution from l'Institut de Médécine et de Chirurgie Expérimentales, Université de Montréal, Montréal, Qué.

[2] *This work was done during the tenure of an American Heart Association Research Fellowship.*

24 hr. after hypophysectomy and the beginning of fasting. Both adrenals were removed and blood samples collected immediately at the time of killing. Adrenal cholesterol was determined by the method of Sperry (7) and the adrenal and plasma ascorbic acid by the method of Roe and Kuether (6).

Results

The results are summarized in Table I and graphically illustrated in Fig. 1.

TABLE I

EFFECT OF ASCORBIC ACID ON ADRENAL CORTICAL RESPONSE TO ACTH

Treatment	Chemical determinations								
	Plasma ascorbic acid, γ/100 ml.			Adrenal ascorbic acid, mgm. /100 gm.			Adrenal cholesterol, mgm. /100 mgm.		
	Time intervals*								
	0	3	6	0	3	6	0	3	6
ACTH**	460 ±98	617 ±79	550 ±104	384 ±13	217 ±9	312 ±6	5.11 ±0.81	3.60 ±0.82	3.78 ±0.18
Ascorbic acid***	460 ±98	1925 ±680	— —	384 ±13	425 ±31	— —	5.11 ±0.81	4.65 ±1.51	— —
ACTH and ascorbic acid	460 ±98	1400 ±248	4000 ±1680	384 ±13	310 ±20	365 ±35	5.11 ±0.81	3.51 ±0.49	3.27 ±0.74

* *Time is expressed in hours after ACTH or sham ACTH administration.*
** *ACTH (Armour, lot No. 60-61), 2 mgm. per 100 gm. of body weight.*
*** *Ascorbic acid (as sodium ascorbate) 200 mgm. per 100 gm. of body weight.*

Plasma Ascorbic Acid

As previously observed (3, 4) ACTH alone caused a slight but transient increase in the plasma ascorbic acid concentration but did not alter the marked elevation resulting from sodium ascorbate administration.

Adrenal Ascorbic Acid

Sodium ascorbate was also observed to increase slightly above normal, the adrenal content of ascorbic acid. When ACTH and sodium ascorbate were both given, the maximum depletion of adrenal ascorbic acid (19%) was significantly smaller than that observed after ACTH treatment alone (44%).

Adrenal Cholesterol

The adrenal cholesterol content was unaffected by sodium ascorbate administration while the depletion following treatment with ACTH or ACTH plus sodium ascorbate was identical.

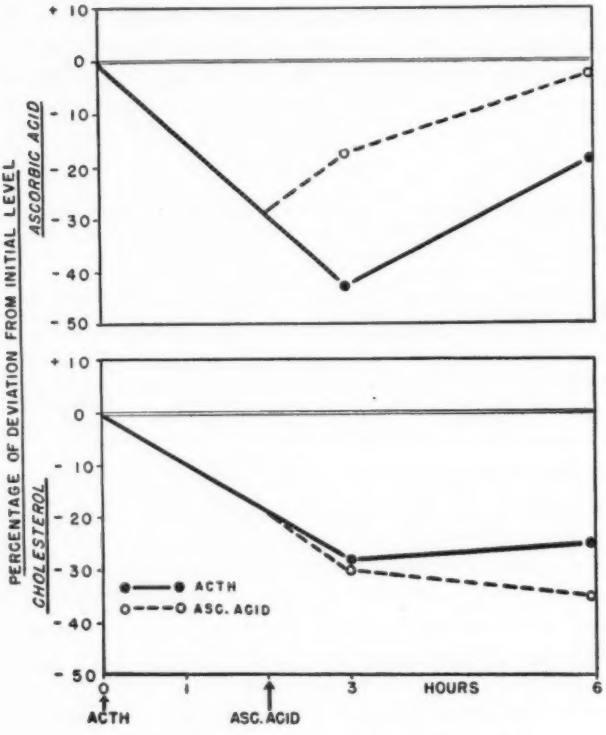

FIG. 1. Plotted in the ordinate are the mean values expressed as % deviation from the hypophysectomized control level. The abscissa indicates time intervals in hours from the moment of ACTH injection.

Conclusions

Elevation of the plasma ascorbic acid level, in the hypophysectomized rat, did not alter the adrenal cholesterol response to ACTH yet partially prevented the adrenal ascorbic acid depletion. This effect is not considered a true conditioning of the adrenal cortical response to ACTH but rather a masking of the normal ascorbic acid decrease by an increased adrenal uptake from the greater quantity available in the plasma. This should be related to Long's observation that in an animal such as the guinea pig which depends on an exogenous source of vitamin C, the ACTH-induced adrenal ascorbic acid depletion is more prolonged than in the rat, which synthesizes its own requirements (5). Both the degree of adrenal ascorbic acid depletion by ACTH and the rate of its repletion would seem to depend on its availability in the plasma.

Summary

The effect of an elevated plasma ascorbic acid level on the adrenal cortical response to ACTH was studied in the hypophysectomized rat. The adrenal cholesterol response to ACTH was unaffected while the ascorbic acid depletion was partially inhibited. These results suggest that the increased adrenal uptake of ascorbic acid from the plasma was sufficient to partially overcome the depleting effects of ACTH.

Acknowledgments

This work was subsidized by a grant from the National Research Council of Canada.

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INFLUENCE DE L'ADMINISTRATION D'ADRÉNALINE ET D'ARTÉRÉNOL SUR L'HYPERTROPHIE DE LA SURRÉNALE AU FROID[1]

PAR ANDRÉ DESMARAIS ET L.-PAUL DUGAL

Abstract

By experiments made on white rats of the Wistar strain, it was shown that:

(1). Injection of either pure adrenaline or pure arterenol has no effect on the hypertrophy of the adrenal cortex during exposure to cold.

(2). Injection of adrenaline lowers the adrenal content of this hormone, without influencing the level of the other hormone, arterenol, in the adrenal medulla.

(3). Injection of arterenol is followed by a drop of this substance in the adrenal medulla, while the adrenaline content remains unchanged.

(4). Injection of adrenaline in rats exposed to a cold environment seems to enhance their survival.

Introduction

Il a été démontré par plusieurs auteurs (5, 6, 8, 9, 11, 12, 14, 15, 16, 17, 18) que l'adrénaline exerce une influence excitatrice sur le système hypophyse-corticosurrénale, à la fois par stimulation du lobe antérieur de l'hypophyse et action directe sur le cortex surrénalien. Sachant, de plus, le rôle important joué par la corticosurrénale au cours de l'acclimatation au froid (1, 2, 3, 7, 10, 13), nous nous sommes demandés si l'administration d'adrénaline ou d'artérénol n'influencerait pas l'hypertrophie du cortex surrénal observée chez les animaux exposés au froid.

Travail Experimental

A. Protocole Expérimentale

Des rats Wistar mâles, pesant de 140 à 160 grammes et recevant eau et Purina ad libitum, ont été divisés en huit groupes. Trois traitements ont été institués, chaque traitement étant administré à deux groupes dont l'un était mis au froid et l'autre gardé à la température normale. De plus, nous avions des groupes non traités, appelés témoins, au froid et à la température normale. Les traitements étaient les suivants:

(1). *Adrénaline*, administrée par voie sous-cutanée en deux injections par jour, chaque injection contenant 0.070 mg. de la substance pure dissoute dans 0.1 cc. d'une solution de NaCl à 0.9%.

(2). *Artérénol*, administré à la même dose et préparé de la même façon que l'adrénaline.

(3). *NaCl, 0.9%*, administré de la même façon et en quantité identique, soit 0.1 cc. par injection.

[1] *Manuscrit reçu le 1 février, 1951.*

Contribution du Département de Recherches en Acclimatation, Faculté de Médecine, Université Laval, Québec, P. Qué., avec l'aide financière du Conseil des Recherches de la Défense nationale du Canada.

Les traitements ont été administrés pendant cinq jours consécutifs et les groupes d'animaux au froid ont été maintenus continuellement à la température de 0° C. Au sixième jour, nous avons prélevé et pesé les surrénales, après quoi nous en avons extrait et dosé l'adrénaline et l'artérénol par la méthode de Euler et Hamberg (4). Ces auteurs ont démontré que leur méthode donnait des résultats équivalents à ceux obtenus par les dosages biologiques. Des dosages d'échantillons de titre connu, ainsi que l'établissement de pourcentages de récupération, nous ont montré que l'erreur expérimentale ne dépasse généralement pas 1.0% lorsque le dosage est fait sur des échantillons dont la concentration est comprise entre 0.2 et 2.0 mg. %.

B. Résultats

Les résultats de cette expérience apparaissent dans le tableau et le graphique ci-joints (Fig. 1). Les pourcentages inscrits au dessus des colonnes du

TABLEAU I

INFLUENCE DE L'ADMINISTRATION D'ADRÉNALINE ET D'ARTÉRÉNOL SUR LE POIDS DES SURRÉNALES ET LEUR TENEUR EN CES DEUX HORMONES*

Traitement	Nombre d'animaux	Adrénaline, µg.	Artérénol, µg.	Poids des surrénales, mg.
Adrénaline au froid	8	1.42 (0.23)**	7.25 (0.99)	34.6
Adrénaline, temp. norm.	7	2.09 (0.35)	9.91 (0.09)	29.7
Artérénol au froid	5	14.70 (0.90)	2.70 (0.15)	33.8
Artérénol, temp. norm.	9	10.43 (1.50)	0.20 (0.01)	27.7
NaCl au froid	17	16.05 (1.67)	7.42 (0.53)	33.3
NaCl, temp. normale	28	11.96 (1.09)	6.89 (0.63)	27.5
Aucun au froid (témoins)	13	10.66 (0.43)	8.30 (0.25)	37.4
Aucun, temp. normale (témoins)	9	7.82 (1.03)	5.83 (0.40)	25.4

* *Les valeurs exprimant la teneur en adrénaline et en artérénol représentent la quantité totale moyenne, en micro-grammes par paire de surrénales.*

** *Entre parenthèses: Ecart type calculé suivant la formule:*

$$S = \sqrt{\frac{\Sigma(d)^2}{n(n-1)}}.$$

graphique indiquent les variations par rapport aux témoins respectifs, soit au froid, soit à la température normale, dont les valeurs sont considérées comme 100. Les constatations suivantes peuvent être faites:

(1). l'administration d'adrénaline abaisse la teneur des surrénales en cette hormone, tandis qu'elle ne modifie à peu près pas la teneur en artérénol;

(2). inversement, l'administration d'artérénol diminue la quantité de cette hormone dans la médullaire sans en changer la teneur en adrénaline;

(3). l'injection du solvant entraîne à elle seule une élévation considérable de la teneur en adrénaline;

(4). le froid, après seulement cinq jours d'exposition, a peu d'influence sur la teneur de la médullo-surrénale en adrénaline et en artérénol;

(5). l'administration d'adrénaline ou d'artérénol est sans influence sur l'hypertrophie de la surrénale au froid.

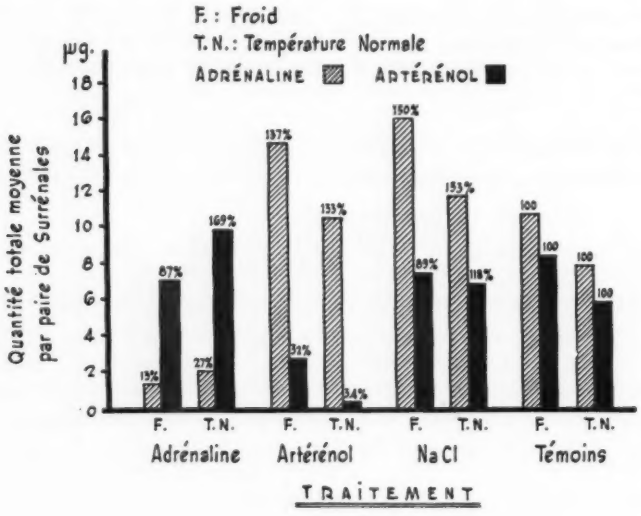

FIG. 1.

C. Interprétation

Les travaux déjà publiés (5, 6, 7, 8, 9, 11, 12, 14, 15, 16, 17, 18) ont démontré sans équivoque l'action déclenchante de l'adrénaline sur la sécrétion cortico-surrénalienne. Bien que nos résultats montrent que l'adrénaline et l'artérénol sont sans influence sur l'hypertrophie corticale au froid, il n'en demeure pas moins vrai que l'administration d'adrénaline a fortement réduit la mortalité entraînée par l'exposition au froid. Voici quel a été le pourcentage de survie pour les quatre groupes d'animaux exposés au froid:

Traitement	Survie
Adrénaline	100.0%
Artérénol	50.0%
NaCl	75.0%
Aucun	36.0%

Les doses d'adrénaline administrées étaient, d'après les travaux déjà mentionnés, suffisantes pour accélérer l'activité corticale; cependant il est possible qu'elles aient été trop faibles pour modifier l'hypertrophie corticale.

Nous croyons plutôt que ce défaut d'action sur l'accroissement pondéral de la surrénale serait dû au fait que la seule exposition au froid est susceptible de déclencher une activation maximale du cortex. Dans ce cas, il faudrait croire que l'effet favorable de l'adrénaline sur la survie des animaux recevant un apport exogène de cette substance serait dû à une meilleure utilisation des hormones corticales.

Nous croyons que les résultats obtenus justifient la poursuite de travaux destinés à vérifier cette hypothèse.

Résumé et Conclusion

L'administration d'adrénaline ou d'artérénol à des rats albinos exposés au froid a révélé les faits suivants:

(1). L'administration d'adrénaline ou d'artérénol est sans influence sur l'hypertrophie de la surrénale au froid.

(2). L'administration d'adrénaline ou d'artérénol abaisse la teneur des surrénales en hormone injectée, sans modifier la quantité de l'autre hormone.

(3). L'administration d'adrénaline semble favoriser la survie des animaux exposés au froid.

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THE LACTIC DEHYDROGENASE ACTIVITY AND PYRIDINE NUCLEOTIDE CONTENT OF HUMAN BLOOD CELLS IN ANEMIA[1]

By M. C. Blanchaer, Pamela Weiss, and D. E. Bergsagel

Abstract

The lactic dehydrogenase activity and the pyridine nucleotide content of the blood cells (PN/BC) of two normal subjects and 12 patients with various degrees of anemia was measured to determine whether the previously reported elevated PN/BC values in anemia are related to an altered metabolic activity of the cells. In 12 well nourished subjects it was found that the PN/BC, expressed in μgm. DPN per gm. of hemoglobin, was elevated in proportion to the severity of the anemia and that this increase was accompanied by a proportional rise in the cellular lactic dehydrogenase activity. The low PN/BC of two poorly nourished patients was accompanied by correspondingly low lactic dehydrogenase levels. The correlation coefficient for the relationship between the lactic dehydrogenase and PN/BC values for all the subjects was + 0.89 and was considered significant since $P < 0.01$. The results suggest that the potential activity of an important enzymatic step in erythrocyte glycolysis is increased in anemia in proportion to the decrease in the hemoglobin concentration and the fall in the hematocrit. The increased PN/BC under these conditions is probably a reflection of an elevated cellular diphosphopyridine nucleotide concentration.

Introduction

It was recently reported (1, 2) that the pyridine nucleotide content of the blood cells (PN/BC) of anemic patients is greater than that of normal subjects. The significance of this finding in relation to blood cell metabolism is not clear since the fluorometric method (3) used in those studies measures the total PN/BC and fails to distinguish between diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN). While both of these substances play important roles as coenzymes in tissue redox reactions, only DPN (4, 5, 6) can function as the hydrogen carrier between the triosephosphate dehydrogenase and lactic dehydrogenase steps of erythrocyte glycolysis.

The present study was undertaken to determine whether the raised PN/BC in anemia is associated with an increase in cellular lactic dehydrogenase activity (LDA). The latter has been used (6) as an approximate index of blood cell DPN content although it also represents the potential activity of an important enzymatic step in erythrocyte glycolysis.

Methods

The subjects were two normal laboratory workers, 11 patients, most of whom suffered from various types of secondary anemia, and one case with both secondary and pernicious anemia. Information pertaining to their clinical condition is given in Table I where the subjects are listed in order of their

[1] *Manuscript received November 16, 1950.*

Contribution from the Nutrition Laboratory of the Department of Physiology and Medical Research, The University of Manitoba, and from the Winnipeg General Hospital, Winnipeg, Canada.

initial hemoglobin concentrations. Most of them had maintained a normal body weight and had consumed adequate amounts of a normal diet for some time prior to the study. Patients 9 and 14, however, showed evidence of being in a poor nutritional state. Patient 9 was emaciated and had eaten little since the acute onset of bronchopneumonia two weeks previously. His total plasma protein concentration was only 3.8 gm. %, with an albumin fraction of 1.5 gm. %. Patient 14 had been in hospital two months for treatment of a chronic periurethral abscess. An investigation of his anemia led to the discovery of the characteristic bone marrow findings of pernicious anemia. Since his red cells were only moderately macrocytic, it was felt that his anemia and anorexia were due to the combined effects of pernicious anemia and chronic infection. The infection and anemia subsequently responded to antibiotic and parenteral liver therapy.

The lactic dehydrogenase activity of the blood cells was determined by the method of Kohn and Klein (6). In this procedure the oxygen consumed by the cells oxidizing lactate in the presence of methylene blue is measured manometrically (11). The oxygen uptake was expressed in μliters consumed in 100 min. at 37.5° C. by the quantity of cells containing 1.00 gm. hemoglobin. Since replicate estimations were not practicable during the course of the investigation, practice determinations were made on the blood of normal subjects to test the precision of the method. The average difference between duplicate results in seven such estimations was 5.1% (S.D. $\pm$ 3.8).

The fluorometric procedure of Levitas *et al.* (7) was used in determining the total PN/BC. The values, calculated as DPN, were expressed in μgm. per gm. of hemoglobin (PN/Hb), and in μgm. per ml. of cells. The details of the hematological methods have been described (1). The statistical methods used are those described by Snedecor (10).

Results

The results shown in Table I and Fig. 1 confirm the earlier reports (1, 2) that a negative correlation exists between the PN/Hb and the severity of the anemia when the latter is expressed as the logarithm of the hemoglobin concentration. Also in agreement with previous work was the finding that the PN/Hb values of the two poorly nourished subjects, 9 and 14, were lower than those of well nourished patients with similar degrees of anemia. Omitting the data on Subjects 9 and 14, the correlation coefficient for the relation between the PN/Hb and logarithms of the hemoglobin values was -0.82. This value was considered significant since $P < 0.01$. A significant but less homogenous relationship was found when the hematocrit readings of the same subjects were plotted against the cellular PN values expressed in μgm. per ml. of cells ($r = -0.60$, $P < 0.05$). In contrast, the amount of PN present in 10^9 red cells was apparently unrelated to the degree of the anemia (Table I).

The estimations were repeated on Subjects 13 and 14 after they had been given oral niacin supplements. Subject 13 received 300 mgm. nicotinic acid

TABLE I

THE LACTIC DEHYDROGENASE ACTIVITY AND PYRIDINE NUCLEOTIDE CONTENT OF BLOOD CELLS IN RELATION TO THE DEGREE OF ANEMIA

Subject	Sex	Age	W.B.C. per cu. mm.	R.B.C. millions per cu. mm.	Hemoglobin, gm.%	Hematocrit	Pyridine nucleotides, μgm. per:			Lactic dehydrogenase activity, μl. O_2 per gm. hemoglobin per 100 min.	Diagnosis
							10^9 RBC	Gm. hemoglobin	Ml. cells		
1	M	23	9225	—	15.5	49.5	—	203	63.6	207	Normal
2	F	66	6600	4.0	13.1	43.6	8.88	271	81.4	219	Peripheral neuritis
3	F	72	5550	5.4	13.0	42.5	6.84	284	87.0	256	Hyperthyroidism
4	F	25	7200	4.8	12.6	42.0	6.62	252	75.6	251	Normal
5	F	70	8650	5.5	12.4	42.5	5.82	258	75.4	270	Diabetes
6	F	29	11100	4.8	12.1	39.6	7.78	308	94.2	286	Chronic nephritis
7	F	62	6225	4.5	11.7	40.2	6.93	266	77.5	252	Portal cirrhosis
8	F	64	8075	4.1	11.0	37.0	6.39	238	70.8	243	Portal cirrhosis
9	M	70	24900	3.3	10.6	37.6	6.62	206	58.1	148	Bronchopneumonia; malnutrition
10	M	20	9800	4.5	10.1	35.5	5.77	257	73.1	263	Hodgkin's disease
11	M	69	14475	4.0	9.6	35.6	7.35	306	82.5	256	Congestive heart failure
12	M	64	9600	3.9	8.4	30.0	6.72	312	87.4	305	Actinomycosis of lung
13*a*	F	17	7800	4.3	6.7	26.8	5.51	354	88.5	348	Menorrhagia
b			11100	4.2	7.6	29.6	7.06	390	100.0	442	Menorrhagia 6 days later
14*a*	M	75	5150	2.2	6.2	21.0	7.04	250	73.8	291	Periurethral abscess; pernicious anemia
b			3825	2.3	5.0	18.3	7.13	328	89.6	320	Periurethral abscess 3 days later

over a six day period following the first analysis. Subject 14 received the same dose in three days. As shown in Table I, the PN/Hb rose in both cases.

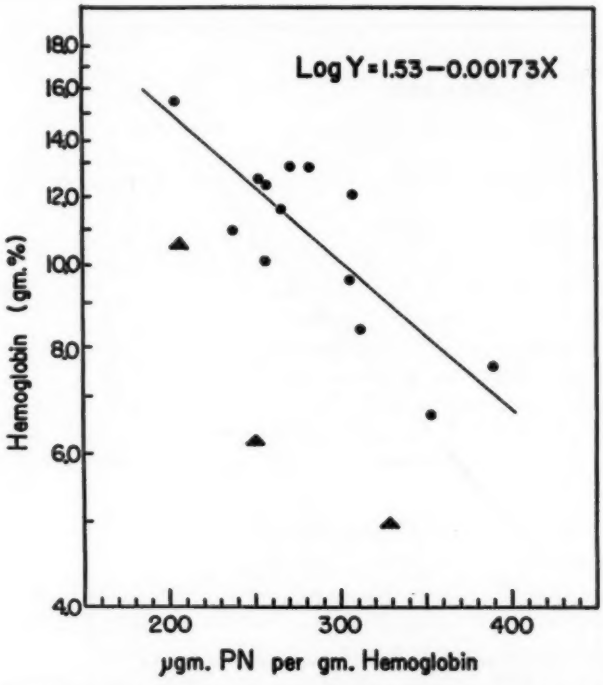

FIG. 1. The relationship between the blood cell PN/Hb and the logarithm of the whole blood hemoglobin concentration. The data on Subjects 9 and 14, marked ▲, were excluded in calculating the regression equation.

The results in Table I and Fig. 2 indicate a positive correlation between the PN/Hb and lactic dehydrogenase activity of the blood cells, including those of Patients 9 and 14. The rise in the PN/Hb which occurred in Subjects 13 and 14 following niacin therapy was accompanied in both cases by an increase in the lactic dehydrogenase activity. The correlation coefficient for the relationship between the LDA and PN/Hb values was +0.89 and was considered significant since $P < 0.01$.

No relation was observed between the PN/Hb or LDA levels and the subjects' age, sex, or leucocyte count.

Discussion

The observations described above, in addition to confirming the earlier reports (1, 2) of a negative correlation between the PN/Hb and the degree of anemia in well nourished subjects, also indicate that the elevated PN/Hr

in anemia is accompanied by a proportional rise in the lactic dehydrogenase activity of the blood cells. Similar conclusions may be drawn from the results when the PN and LDA values are calculated per milliliter of cells,

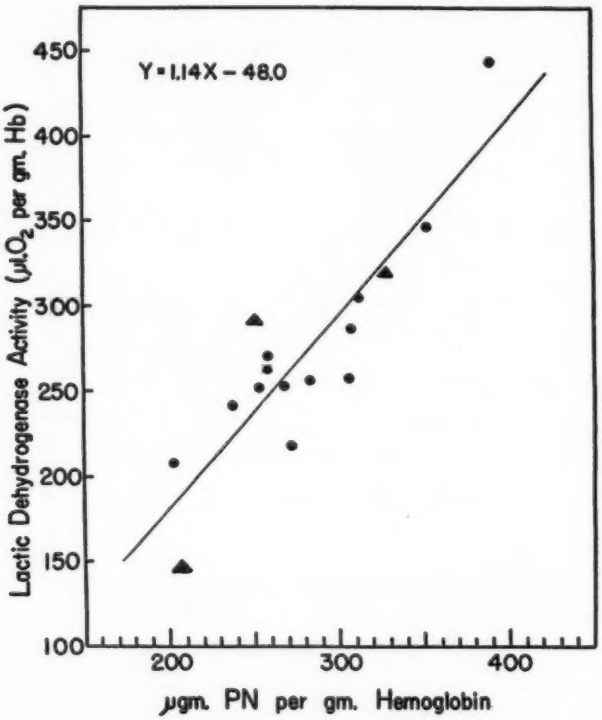

FIG. 2. The relationship between the lactic dehydrogenase activity and the blood cell PN/Hb. The data on Subjects 9 and 14, marked ▲, were included in calculating the regression equation.

but not when they are expressed per red cell. It seems possible therefore that the PN content and the lactic dehydrogenase activity of the individual blood cells remains relatively constant in anemia in spite of moderate differences in the mean corpuscular volume. Since the present group of subjects is small and includes no uncomplicated cases of primary macrocytic anemia, it seems desirable to withhold judgment on this aspect of the data until more material is available. It may be noted, however, that the results reported here support our earlier conclusion (1) that the cellular *concentration* of the pyridine nucleotides, calculated in the conventional unit of μgm. PN per ml. of blood cells, is elevated in anemia.

The extent to which variations in LDA reflect red cell metabolism is not clear since the reactions involved in the measurement of LDA in the presence of methylene blue are not well understood. The following is one of a number

of schemes (4, 5, 8, 9) which have been offered to explain the role of DPN, methylene blue, and lactic dehydrogenase apoenzyme in the oxidation of lactate to pyruvate by blood cells.

$$2\ CH_3CHOHCOOH + 2\ DPN \xrightarrow{\text{apoenzyme}} 2\ CH_3COCOOH + 2\ DPN \cdot H_2$$
$$2\ DPN \cdot H_2 + 2\ MeB \longrightarrow 2\ DPN + 2\ MeB \cdot H_2$$
$$2\ MeB \cdot H_2 + 2\ O_2 \longrightarrow 2\ MeB + 2\ H_2O_2$$
$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

Net: $2\ CH_3CHOHCOOH + O_2 \longrightarrow 2\ CH_3COCOOH + 2\ H_2O$

Since LDA is dependent upon the net activity of a number of successive reactions it seems probable that the oxygen uptake is only an approximate indication of the cellular DPN concentration. Nevertheless the increase in LDA in anemia is itself of interest since it indicates that such cells can oxidize DPN . H_2 and reduce pyruvate more rapidly than normal blood cells. Although the exact significance of this phenomenon in anemia is not apparent, the change may be of importance in the general economy of the cells since the lactic dehydrogenase system is linked through DPN to the triosedehydrogenase enzyme which is thought (3) to regulate the overall rate of erythrocyte glycolysis.

Acknowledgments

The work reported in this paper was supported by a grant from the Medical Division of the National Research Council (Canada), and was performed while MCB was a Markle Scholar in Medical Science. Dr. Dorothy E. Jefferson kindly made the blood cell counts. Merck and Company and Ayerst, McKenna, and Harrison Limited donated generous supplies of reagents and vitamins.

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PEPTIDASE ACTIVITY IN HUMAN SERUM UNDER VARIOUS PATHOLOGICAL CONDITIONS, IN PREGNANCY, AND FOLLOWING THE ADMINISTRATION OF ACTH

By A. M. Cullen, V. T. Barber, M. K. Birmingham, and K. Stern

Abstract

A study was made of the serum peptidase activity of 37 normal control subjects, of 80 subjects suffering from various pathological conditions, of eight normal subjects following administration of ACTH, and of one case of normal pregnancy. The substrate used was *l*-leucylglycylglycine and in some cases glycylglycylglycine. The highest values for peptide hydrolysis were observed in cases of lymphatic leukemia. Normal, or close to normal, values were found in the serum of patients with aleukemic lymphatic leukemia and with myelogenous leukemia. In a case of Hodgkin's disease and in four out of six cancer patients the peptidase activity was elevated depending on the severity of the disease. Normal activity was observed in a case of multiple myeloma and nonmalignant tumor. In five out of 11 patients suffering from tuberculosis the peptidase activity of the serum was high. In most cases of acute infection the peptidase activity was elevated, in cases of mild respiratory infections the activity was within or close to the normal range. There was no clear-cut relationship between the cellular composition of the blood and the serum peptidase level. Injection of ACTH usually had little or no effect on the serum peptidase level of normal subjects. No change in serum peptidase activity was observed in a subject during the course of a normal pregnancy. The implication of these findings is discussed.

Introduction

The question of the origin and significance of serum peptidases has aroused widespread interest. Increases in serum peptidase activity have been demonstrated in cases of fever (4), burns (18), acute infection (1), bone fracture (15), tourniquet shock (2), and experimental hemolytic anemia (13). Divergent results have been reported on the activity of serum peptidases following ACTH administration (5, 10, 11, 12), which is known to cause the dissolution of lymphocytes and lymphoid tissue. It has been shown that erythrocytes (11, 8, 6, 14) and leucocytes (14) have a high content of enzymes capable of peptide hydrolysis. In the present work the serum peptidase activity has been studied under various conditions involving in some cases marked changes in the cellular composition of the blood, and variations in hormone levels.

Methods

The study was made on the serum of 36 normal subjects and of 80 patients, including six fracture cases, eight pneumonia cases, seven asthma cases, six carcinoma, 11 tuberculosis subjects, and 37 patients with various other conditions. The serum peptidase level was also studied in one patient during the

[1] *Manuscript received February 13, 1951.*

Contribution from the Gerontological Unit, Department of Psychiatry, McGill University Montreal, Que. This work was aided by a grant from the National Research Council of Canada and by a grant from the John and Mary R. Markle Foundation.

course of recovery from severe barbiturate poisoning, in eight normal control subjects following intramuscular administration of adrenocorticotrophic hormone, and in one subject during the course of normal pregnancy.

Nonfasting blood was obtained from the cubital vein and the serum separated by centrifugation as soon as clotting was complete. *l*-Leucylglycylglycine (LGG), and in a few cases glycylglycylglycine (GGG), were used as substrates. The substrate concentration was 0.05 molar and 0.001 *M* cobalt sulphate was used as activator. Hydrolysis was measured by the microtitration method of Grassmann and Heyde (4). The determinations were carried out, and the data compiled, as described in previous publications (1, 15). The reaction proceeded in a water bath at 39° C. and at pH 7.8 for the hydrolysis of LGG and at pH 7.0 for the hydrolysis of GGG. The results were expressed as % per hour hydrolysis of tripeptide (based on the complete splitting of one peptide linkage) for 0.2 ml. of serum per ml. of test solution. Care was taken to avoid haemolysis of serum.

In most cases total white blood cells, differential counts, and sedimentation rates were determined. These determinations were made by the standard haematologic methods, and the differential counts were done on 200 cells.

Results

Fig. 1 is a scattergraph showing the rate of hydrolysis of LGG by the serum of normal subjects and of patients suffering from a variety of pathological conditions. The rate of hydrolysis by normal serum ranges from 5.5 to 8.5% per hour. The highest rates are found in cases of lymphatic leukemia. In four out of six of the carcinoma cases studied the peptidase activity was elevated. The highest values in this group were obtained in two advanced cases, one case of bronchogenic carcinoma, and one of carcinoma of the stomach. In a case of multiple myeloma and nonmalignant tumor the rate was normal. Most cases of acute infection showed a peptidase activity above the normal level. In one case of mastoiditis and meningitis the serum peptidase activity was low. In this case there was a very high content of peptidases in the cerebrospinal fluid (16). In five out of 11 patients suffering from tuberculosis, the peptidase activity was elevated. One advanced case with extreme emaciation showed a level of 26% per hour. Cases of mild respiratory infections were within or close to the normal range. The activity was high in all cases of fracture and in some asthma cases. In mental disturbances three out of eight patients showed a slightly increased activity of serum peptidase. Two patients with hypertension showed an increased activity.

In Table I the serum peptidase activity of 77 patients together with the lymphocyte and leucocyte count and the sedimentation rate are listed. Statistical analysis showed no correlation between the cell counts or the sedimentation rate and the serum peptidase activity. In the three cases of lymphatic leukemia, where the total white blood cells and absolute lymphocyte

count were abnormally high, the serum peptidase activity was also high. In one case of myelogenous leukemia and two cases of aleukemic lymphatic leukemia the serum peptidase activity was within or near the normal range.

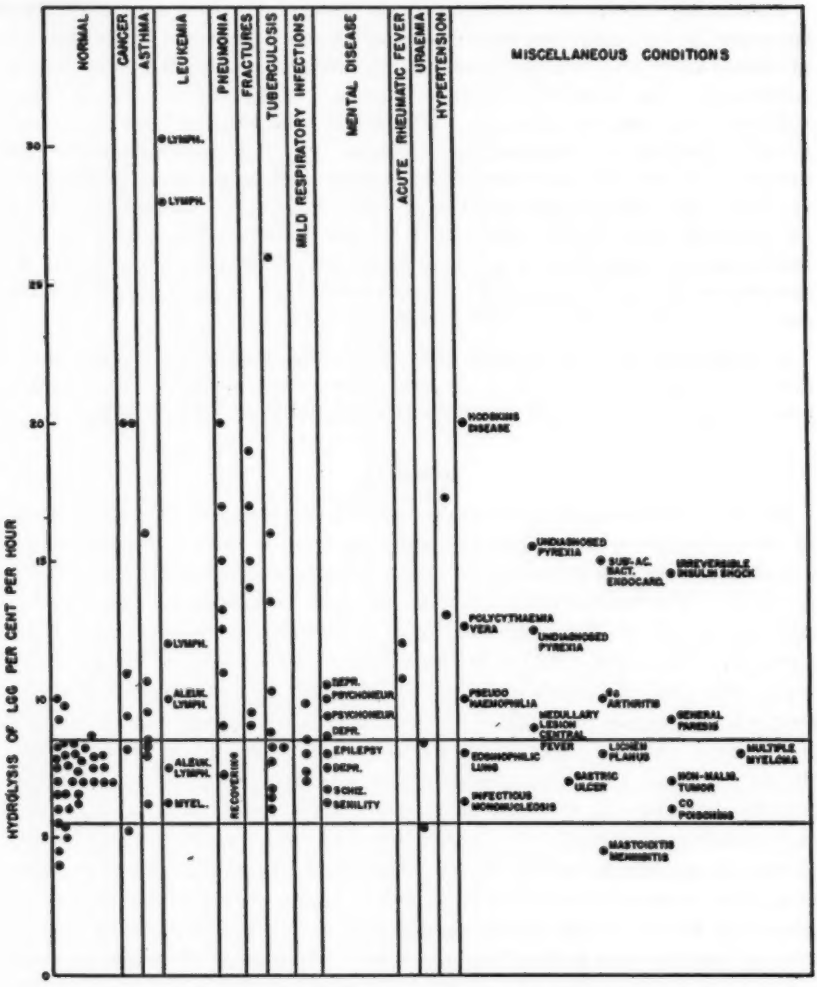

FIG. 1. The rate of hydrolysis of LGG by the serum of normal subjects and of patients suffering from a variety of pathological conditions.

In a case of infectious mononucleosis, with a high percentage of atypical lymphocytes, the hydrolysis of LGG by serum was normal. The serum of another patient with infectious mononucleosis (not listed in Table I) hydrolyzed GGG at a rate of 12% per hour. This is within the normal limits for

TABLE I

SERUM PEPTIDASE ACTIVITY, LEUCOCYTE COUNTS, AND SEDIMENTATION RATES IN PATIENTS SUFFERING FROM VARIOUS PATHOLOGICAL CONDITIONS

Name	Diagnosis	Sex	Age	Leucocytes per cu. mm.	Lymphocytes and monocytes per cu. mm.	Granulocytes per cu. mm.	Corrected sed. rate, mm. per hr.	% per hr. hydrolysis of LGG
Ge	Lymphatic leukemia	M	33	357,000				28
In	Lymphatic leukemia	M	71	75,000	72,750	2250		12
Yo	Lymphatic leukemia	F	77	705,000	648,600	56,400		16
Ag	Aleukemic lymphatic leukemia	F	55	1350	926	424	41	10
Mo	Aleukemic lymphatic leukemia	M	47	6500	4680	820		7
Fi	Myelogenous leukemia	M	55	16,400				7
Mar	Hodgkin's disease	M	22	4000			18	21
Fa	Vulvar carcinoma	F	39				42	9
Mi	Bronchogenic carcinoma—June 3 June 7	M	53	3900 8600	1599 4152	2401 4448	23 50	20 13
St	Cervical carcinoma	F	52	10,300			55	11
Th	Cervical carcinoma	F	47	9000			30	5
Ja	Carcinoma of mouth	M	50				32	10
Fie	Tuberculosis, two years	M	17	8950				9
Ko	Tuberculosis	F	25	10,050				8
La	Tuberculosis	M	20	10,450	3031	7419	32	14
Ni	Tuberculosis	M	49	9440				10
Re	Tuberculosis	F	48	7750				7
Ru	Tuberculosis	M		7350	1176	5174	2	6
Ea	Pneumonia	M	43	27,000				14
Ma	Pneumonia	M	60	9050			34	9
Mon	Pneumonia	F	81	18,400				17
Pr	Pneumonia	M	40	11,000				15
Ro	Pneumonia	F	29	6750	1958	4792	41	13
Sm	Influenza pneumonia	M	41	12,200	4636	7564	10	20
Su	Staphylococcus pneumonia	F	55	25,850	5170	20,680	50	11

TABLE I—*Concluded*

SERUM PEPTIDASE ACTIVITY, LEUCOCYTE COUNTS, AND SEDIMENTATION RATES IN PATIENTS SUFFERING FROM VARIOUS PATHOLOGICAL CONDITIONS

Name	Diagnosis	Sex	Age	Leucocytes per cu. mm.	Lymphocytes and monocytes per cu. mm.	Granulocytes per cu. mm.	Corrected sed. rate, mm. per hr.	% per hr. hydrolysis of LGG
Am	Subacute bacterial endocarditis	F	25	9700	2425	7275	38	15
Ba	Gonococcal arthritis	M	26	6500	5100	1400		10
Br	Acute rheumatic fever	M	26	9000	2970	6030	5	11
Ot	Acute rheumatic fever	F	27	10,400	4368	6032	32	12
Co	Mild respiratory infection	F	27				22	10
Wi	Mild respiratory infection	F	28	7100			22	9
Fis	Asthma	F	49	7000			33	10
Kn	Asthma	F	53	12,700			38	11
La	Asthma	F	33	8000			8	6
Lee	Asthma	M	51	7550			32	8
Mn	Asthma	M	45	11,800	2950	8850	34	9
Wil	Asthma	M	64	13,600	4080	9520		12
Mor	Acute purulent mastoiditis and meningitis	F	54	21,900				5
As	Undiagnosed pyrexia	M		8200	4018	4182	5	15
Da	Lesion at base of medulla	F	29	8600			35	9
Kl	Infectious mononucleosis	M	17	5800	3480	2320		6
Ja	Eosinophilic lung	M	20	57,000				8
Cu	Uraemia (extremely emaciated)	M	59				25	5
For	Uraemia (creatinine 14.8 mgm. %)	F	47				16	9
Fo	Multiple myeloma	M	55	21,500	9675	11,825	15	7
Bu	Nonmalignant tumor (uterine)	F	46	10,500			49	6
Ke	Irreversible insulin shock	F	26	20,800	2496	17,304		15
Ca	Carbon monoxide poisoning	F	32	26,200	2096	24,104	16	6
You	Polycythaemia vera R.B.C. 6,580,000 per cu. mm.	F	40					13

the hydrolysis of GGG.* One patient with an eosinophilic lung, with a white blood cell count of 57,000 cells per cu. mm. (mostly eosinophils) had a normal serum peptidase activity. In a case of polycythaemia vera, where the red blood cells numbered more than six million per cu. mm., the LGG splitting capacity of serum was increased. A patient suffering from Hodgkin's disease had a white blood cell count of only 4000 per cu. mm., while his serum peptidase activity was elevated to 18% per hour. There is thus no consistent relation between the cellular composition of the blood and peptidase activity.

In Table II is shown the hydrolysis of GGG by serum during the first 259 days of pregnancy. The rate remained at a normal level throughout. By the 259th day the white blood cells and sedimentation rate were definitely higher than normal.

TABLE II

THE HYDROLYSIS OF GGG BY SERUM DURING PREGNANCY
(THE SUBJECT WAS A HEALTHY WOMAN AGED 30)

Day of pregnancy	Leucocytes per cu. mm.	Lymphocytes and monocytes per cu. mm.	Granulocytes per cu. mm.	Corrected sed. rate, mm./hr.	Hydrolysis of GGG, % per hr.
Prior to pregnancy					9
48th	13,850	2993	11,357	0	9
62nd					10
92nd	11,900	2499	9401	6	8
119th	17,500	4375	13,125	0	8
19th				6	9
259th	22,700	5675	16,025	24	9

In Table III is shown the effect on the hydrolysis of LGG and GGG by serum when adrenocorticotrophic hormone was administered intramuscularly to a group of eight normal adult subjects. In five out of eight cases there appeared to be an increase in peptidase activity around four hours after the injection was administered. With the exception of one case in which the activity was nearly doubled (Na) the increases were small, and the results were not statistically significant.

In Table IV are shown the levels of serum peptidase in a patient recovering from acute barbiturate intoxication. The patient (Wi) was a man of 33 years who had swallowed 50 or more grains of phenobarbital. He remained unconscious for 14 days and had an increased body temperature until the 22nd day. The peptidase activity was extremely high the first day after the drug had been taken and gradually decreased to a nearly normal value by the 34th day.

** The rate of hydrolysis of GGG has been determined using the serum of 14 normal adults. The average rate in these subjects was found to be 10% per hour, ranging from 7 to 12% per hour.*

TABLE III

THE HYDROLYSIS OF LGG AND GGG BY SERUM FROM HEALTHY ADULTS FOLLOWING THE INTRAMUSCULAR ADMINISTRATION OF ACTH

Name	Age	Sex	W.B.C. per cu. mm.	Lymphocytes		Hr. after first injection	Hydrol. of LGG % per hr.	Hydrol. of GGG % per hr.	Remarks
				% of W.B.C.	Per cu. mm.				
Ap		F	7700			0		8	18 mgm. ACTH (single dose)
			12,500	17	2125	3½		8	
			16,900	23	3887	5½		10	
						7½		9	
Be	34	F	8150	49	3593	0	6		26 mgm. ACTH (single dose)
			6700	40	2680	1	6		
			8300	22	1826	2	7		
			17,652	14	2471	4	10		
Ch	24	F				0	7		30 mgm. ACTH (single dose)
						1½	5		
						2½	5		
						4½	6		
						6½	4		
Na	38	F	7450	43	3203	0	8		25 mgm. ACTH (single dose)
			8050	36	2898	1	9		
			8700	26	2262	2	11		
			22,000	8	1760	4	15		
			23,800	9	2142	6	12		

TABLE III—*Concluded*

THE HYDROLYSIS OF LGG AND GGG BY SERUM FROM HEALTHY ADULTS FOLLOWING THE INTRAMUSCULAR ADMINISTRATION OF ACTH—*Concluded*

Name	Age	Sex	W.B.C. per cu. mm.	Lymphocytes		Hr. after first injection	Hydrol. of LGG % per hr.	Hydrol. of GGG % per hr.	Remarks
				% of W.B.C.	Per cu. mm.				
Fr		M				−24	6		420 mgm. ACTH (70 mgm. every 6 hr.)
						0	4		ac breakfast
						24	5		pc breakfast
						25	5		pc supper
						36	5		ac breakfast
						48	6		
Go	29	M				0	12		420 mgm. ACTH (70 mgm. every 6 hr.)
						4	13		
						8	14		
						12	12		
						24	12		
Gr	32	M	6800	51	3468	−1	5		25 mgm. ACTH (single dose)
						¾	5		
						1¼	5		
			9340	18	1684	3¾	5		
Sh	22	M				0	5		240 mgm. ACTH (60 mgm. every 4 hr.)
						4	8		
						8	6		
						12	8		

TABLE IV

SERUM PEPTIDASE ACTIVITY OF A MAN RECOVERING FROM BARBITURATE POISONING

Days after taking about 50 grains phenobarbital	% per hr. hydrolysis of LGG	W.B.C. per cu. mm.	Sedimentation rate
1	23	17,800	20
12	19	36,000	34
15	16		
26	12	10,350	35
34	9		

Discussion

Peptide splitting enzymes are known to be present in a variety of tissues (18, 3, 9, 7), as well as in the cellular components of the blood (11, 8, 6, 14). The most plausible conclusion to be drawn from the results presented seems to be that pathological conditions which are associated with cellular damage result in a high serum peptidase activity, apparently due to a leakage of these enzymes out of the cells.

Since adrenocortical hyperactivity is known to be associated with exposure to traumatic stimuli, one might assume a direct interrelation between high serum peptidase activity and adrenocortical activity. One of the mechanisms by which such an interdependence could be brought about has been suggested by Holman, White, and Fruton (5). These authors believe that the main source of serum peptidases might be the lymphatic cells and ascribe the marked rise they observed in the serum peptidase levels of rats following ACTH administration to the destruction of lymphatic cells caused by this hormone. However, our work indicates that the increase in serum peptidase of normal subjects, some of whom had received as much as 420 mgm. of ACTH, is variable and may be absent altogether. Further, during the course of pregnancy the serum peptidase level remained constant although urinary glucocorticoid excretion is known to double in the first trimester, return to normal by the 100th day, and increase to about 400% of normal during the seventh and eighth month of gestation (17). This seems to indicate that there is no clear-cut dependency of serum peptidase activity upon adrenocortical function. These observations and their interpretation are in line with those of Schwartz and Engel (11), who believe that "plasma LGGase activity reflects changes in clinical status and is not necessarily mediated through the adrenal cortex". The authors found no significant change from normal in the serum peptidase level of rats following ACTH injection (in contrast to the results of Holman *et al.* (5)) and obtained normal values in patients with Cushing's and Addison's disease.

There appears to be no obvious relationship between the cellular composition of the blood and the serum peptidase level. It is true that in lymphatic leukemia patients with white counts over 350,000, the activity of the

serum was extremely high. However, in one case of lymphatic leukemia the white count was 75,000 and the peptidase activity elevated only moderately. Conversely, in many conditions which did not show a marked alteration of lymphocyte or granulocyte count increased peptidase activity occurred.

These results do not exclude the possibility that the formed elements of the blood contribute to the serum peptidase level. The work of Smith *et al.* (13) shows that experimentally induced hemolytic anemia raises the serum peptidase activity of dogs. To assess the extent to which the different types of blood cells are a source of these enzymes in the serum a knowledge of the rate of turnover of the blood cells under various conditions would be of greater importance than the actual cell count.

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THE EFFECT OF ASCORBIC ACID ON EXPERIMENTAL NEPHRITIS[1]

By Frederick F. Howatt and Peter W. Davey

Abstract

This is a report of the effect of ascorbic acid in massive doses on experimental acute glomerulonephritis. The nephritis is induced by injection of bovine gamma globulin into rabbits, and the resultant lesions closely resemble the acute glomerulonephritis of man. There was a greater weight loss during the experiment in the treated animals. The renal lesions and precipitin titers of the controls and treated animals were not significantly different. Nevertheless there was a suggestion that the treated animals were more severely affected than the controls. The microscopic blood picture was unchanged in the two series, but the blood sugar was moderately higher in the controls.

Introduction

Recently a number of reports have appeared in the literature dealing with the use of massive doses of ascorbic acid in the therapy of acute rheumatic fever (6), and rheumatoid arthritis. These diseases belong to the group in which hypersensitivity (9) may play an important role. It is said that vitamin C deficiency may be an additional factor in the pathogenesis of acute rheumatic fever (8).

This is a preliminary report of a study of the effect of massive doses of ascorbic acid on experimental acute glomerulonephritis induced in rabbits by repeated injections of bovine gamma globulin. At the same time the effect of such therapy on the Arthus phenomenon, blood picture, and precipitin antibody titers has been studied.

Procedure

Three weeks after unilateral nephrectomy, 16 rabbits were each given a sensitizing dose of 20 cc. of 10% bovine gamma globulin. The animals, which weighed between 2260 and 3850 gm., were divided into two groups of eight each. The first group served as controls, while the second group received 250 mgm. of ascorbic acid* intramuscularly at 12 hr. intervals, commencing 12 hr. prior to the sensitizing dose. The controls and the treated animals were given a desensitizing dose of 1 cc. of 10% bovine gamma globulin 10 days after the sensitizing dose, followed in 24 hr. by a shocking dose of 20 cc. of 10% bovine gamma globulin. The animals were all sacrificed on the 18th day and autopsied immediately.

During the test period, the daily intake of food was carefully measured. The animals were weighed before, during, and at the end of the experiment. Venesection was carried out before the sensitizing dose, on the 10th day and again just before the animals were sacrificed. Ten cc. of blood were

[1] *Manuscript received February 23, 1951.*

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* *"Cenolate" (methylglucamine ascorbate, Abbott) supplied through the courtesy of the Abbott Laboratories Limited, Montreal.*

removed on each occasion. Estimation of the W.B.C., differential leucocyte count, blood sugar, and the precipitin antibody titer of the serum was carried out on each blood sample. Forty-eight hours before sacrificing, each animal received three intracutaneous injections, one of 0.1 cc. of 10% bovine gamma globulin, one of 0.1 cc. of 0.01%, and one of 0.1 cc. of 0.001% solution. The resulting skin reactions were read at 24 hr. and again at 48 hr.

At autopsy, the paired adrenals, liver, spleen, and the remaining kidney were weighed. All organs were fixed in Zenker–Formol, sectioned at 5μ, and stained with hematoxylin and eosin. In addition, sections of the kidneys were sectioned at 3 μ and stained with periodic acid Schiff reagent and Masson's trichrome stain.

Results

One animal from each group died early in the course of the experiment from causes unrelated to this study.

Renal lesions were found in all animals of both groups. They were graded in severity from $\pm$ to ++++ depending on the presence of microscopic glomerular changes. These included the formation of epithelial crescents; proliferation and swelling of the epithelium and endothelium of the tuft; thickening and splitting of the glomerular basement membranes; hyaline thrombi in glomerular capillaries; and swelling of the glomeruli. These results are tabulated below.

The precipitin antibody titer as estimated on the various blood samples may be seen in the table.

All animals received an adequate diet and the average food intake was well maintained except for the day immediately following the desensitizing dose when there was a moderate fall.

All the animals lost weight during the experiment and the individual losses are seen in the table. The average weight loss of the control group was 130 gm., whereas that of the treated group was 275 gm.

The Arthus phenomenon read at 24 hr. and again at 48 hr. is tabulated for each animal with the injection of the 10% solution being used as an example. The results are classified from $\pm$ to ++++ according to the amount of redness, swelling, and induration present. No appreciable difference between the test animals and the controls could be detected.

The results of the blood sugars taken at the various time intervals throughout the experiment may be seen in the table. There is a suggestion that the final values for the controls were higher than those for the animals receiving ascorbic acid.

The W.B.C. done on each sample and the differential leucocyte count showed no significant change from the normal values (2), during or at the conclusion of the experiment.

The weights of the various organs were not significantly different in either the control group or in the group treated with ascorbic acid.

TABLE I

TABULATION OF RENAL LESIONS, PRECIPITIN TITERS, WEIGHT LOSS, ARTHUS PHENOMENON, AND BLOOD SUGARS OF NEPHRITIC RABBITS, TREATED WITH MASSIVE DOSES OF ASCORBIC ACID, AND OF CONTROLS

	Animal No.	Renal lesions	Pptin. titers	Weight loss	Arthus phenomenon		Blood sugar
					24 hr.	48 hr.	
Group A controls	1	++++	1/1000	275 gm.	+++	++++	132 mgm. %
	2	++	1/10000	80 gm.	++	+++	130 mgm. %
	3	±	1/1000	185 gm.	++	++	110 mgm. %
	4	+	1/100	215 gm.	++	+	135 mgm. %
	5	Died	Died	Died	Died	Died	Died
	6	+	1/5000	15 gm.	+++	++	205 mgm. %
	7	+	1/100	40 gm.	+++	++	148 mgm. %
	8	++	1/10000	105 gm.	++	+	110 mgm. %
Group B, treated with vitamin C	9	+	1/10	130 gm.	++	++	117 mgm. %
	10	++	1/10000	480 gm.	++	+	108 mgm. %
	11	++	1/5000	350 gm.	+++	++++	115 mgm. %
	12	++++	1/5000	50 gm.	++	++	110 mgm. %
	13	+	1/1000	265 gm.	++	++	124 mgm. %
	14	+++	1/1000	300 gm.	+	+	124 mgm. %
	15	Died	Died	Died	Died	Died	Died
	16	±	1/1000	350 gm.	++	++	124 mgm. %

Discussion

The renal lesions produced by this method, which was first devised as an experimental tool by Rich and Gregory (7), resemble acute glomerulonephritis in man. In this experiment there is no significant difference in the renal lesions of animals receiving massive doses of ascorbic acid and in lesions seen in the controls. The former, however, appear if anything to be slightly more severe. Dugal and Therien (3) state that vitamin C protects the kidneys of rats against renal changes and nephromegaly dûe to cold stress. It would seem then that stress occasioned by the antibody–antigen reaction in this experiment was unaffected by vitamin C in massive doses.

In this small series, there is no significant difference between the precipitin antibody titers in test and control animals. Other investigators have found antibody levels to be affected by vitamin C administration. There is suggestive evidence in this experiment that those animals receiving massive ascorbic acid therapy had higher titers than the controls. Enhancement of antibody formation with excess ascorbic acid administration has been reported by Madison and Manwaring and Jusatz (5) in rabbits, but other authors, notably Hochwald and Schwartz (4) found no increase in antibody production in guinea pigs immunized with horse serum and treated with excess doses of vitamin C. It is apparent from the conflicting evidence in the literature that a much larger series of animals would be necessary to decide the exact role of excess ascorbic acid in precipitin antibody production.

PLATE I

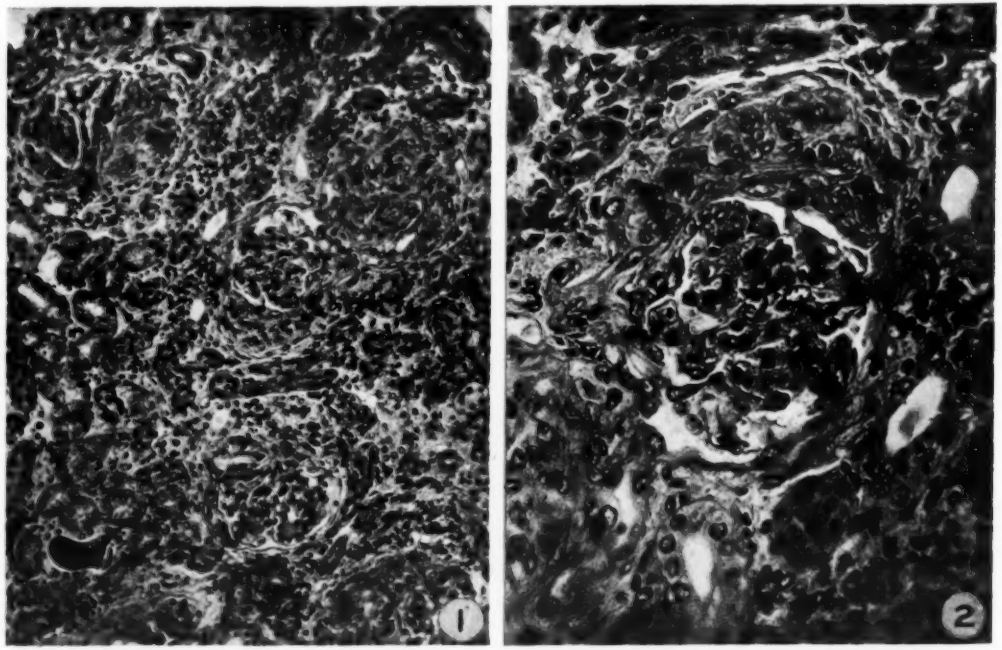

FIG. 1. Kidney from rabbit No. 12, treated with ascorbic acid. Glomeruli showing marked crescent formation, capillary epithelial and endothelial proliferation, with infiltration of interstitial tissue with lymphocytes. ×130.

FIG. 2. Kidney from rabbit No. 12 treated with ascorbic acid. Glomerulus showing crescent formation, proliferation of capillary endothelium and epithelium. ×343.

The Arthus phenomenon too, was not significantly affected by ascorbic acid administration although a comparable test, the tuberculin reaction, is said to be modified by this therapy (1).

There was a greater weight loss in treated animals, but we are unable to account for this except to state that these animals too, seemed to have more severe renal lesions and higher precipitin titers.

We feel that massive ascorbic acid administration has no appreciable effect on the lesions of acute glomerulonephritis as produced by the method outlined. However, because of the impression that the renal lesions were slightly worse and the precipitin titers slightly higher in the treated series, a further experiment is being done, in which the time at which the animals are sacrificed following the shock dose will be greatly extended. In this way, the effect of ascorbic acid on a more chronic type of nephritis is being studied.

Conclusion

1. Massive doses of ascorbic acid had no significant effect on the lesions of experimental glomerulonephritis.

2. The precipitin antibody titer coincident to the nephritis was not appreciably affected by ascorbic acid administration.

3. There is no effect on the blood picture, weight of organs, or Arthus phenomenon in animals treated with ascorbic acid in massive doses.

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STUDIES ON THE RELATIONSHIP BETWEEN VIRUS AND HOST CELL

II. THE BREAKDOWN OF T2r^+ BACTERIOPHAGE UPON INFECTION OF ITS HOST *ESCHERICHIA COLI*

By S. M. Lesley, R. C. French, A. F. Graham, and C. E. van Rooyen

Abstract

Infection of *E. coli* with P^{32} labelled T2r^+ bacteriophage resulted in the conversion of a part of the isotope to a form soluble in 5% trichloroacetic acid. This acid soluble phosphorus was used as a measure of the breakdown of the virus. When the phage to cell ratio was less than unity a two step breakdown curve was obtained corresponding to the two cycles of infection in this type of experiment. The heights of the steps represented 5 and 32% breakdown, the second rise commencing after about 25 min. Multiple infection of cells yielded a curve which rose without inflection to about 23% breakdown at 20 min. after which there was little further increase. When the addition of radioactive phage was preceded by sufficient nonradioactive phage to infect most of the cells in the culture, the amount of breakdown of labelled virus increased to about 55% within 20 min. at which time breakdown practically ceased. Control experiments indicated that dead phage in the labelled preparations contributed little to the acid soluble P^{32} fraction in these experiments and that the breakdown resulted from infection of the cells by virus. It is suggested that infection of cells by phage stimulates some mechanism whereby phage adsorbed to the cell at a later time is broken down extensively at the cell surface. The experimental results are interpreted in the light of this concept.

Introduction

The purpose of this work was to investigate some of the phenomena connected with the apparent disappearance of the virus particle following infection of its host. That some extensive alteration does take place in virus structure shortly after infection has already been suggested by many workers. Thus studies by Hoyle (8) and by Henle *et al.* (7) on influenza virus indicated that once adsorbed to the host cells the virus could not be recovered by rupture of such cells until several hours after infection. Similar behavior of other viruses such as those of vaccinia, ectromelia, and psittacosis has led to the suggestion by Bauer (1) that the most probable change in the virus seems to be "the dissolution of the virus body into a soluble phase which then diffuses through a considerable volume of cytoplasm and organizes it into virus material by an action upon the chromidia and their enzymes".

Certain bacteriophages appear to undergo a somewhat analogous change. By breaking open the cell at different times after the infection of *Escherichia coli* with some phages of the T group, Doermann (6) has demonstrated that no virus could be detected until about the middle of the "latent period". In a recent review Luria (13) has correlated this finding with those obtained

[1] *Manuscript received January 16, 1951.*

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Aided by a grant from the National Cancer Institute of Canada.

in genetic and other studies on bacteriophage infection. He has suggested that infection produces a reorganization of the cell, the virus particle being divided into a number of genetic units. Following replication of these units they are reorganized into mature phage particles. Direct evidence that some physical breakdown of the infecting virus particle does occur is given by Putnam and Kozloff (14). These workers found that on lysis of cells infected with P^{32} labelled T6r^+ bacteriophage the greater part of the isotope was found in the medium as low molecular weight phosphorus. A significant amount of the isotope appeared in the newly formed virus.

While the explanation advanced by Luria (13) to account for the apparent disappearance of the infecting bacteriophage is an attractive one, it is based to a large extent on indirect evidence. In an attempt to throw more light on the mechanism of infection we have carried out experiments with T2r^+ bacteriophage labelled with P^{32}. Preliminary work revealed that shortly after infection of the cells P^{32} appeared in the medium in a form soluble in 5% trichloroacetic acid. This suggested a rapid breakdown of the infecting virus. Thus, using the formation of acid soluble P^{32} as a measure of breakdown, the changes occurring in cells infected under different experimental conditions were investigated. The results which have been summarized in a preliminary paper (10) are now described in detail.

Materials and Methods

Bacteriophage Preparations

The growth, purification, and properties of T2r^+ bacteriophage labelled with P^{32} have been reported in detail (11). Purified phage was kept at 5° C. as a suspension in 0.85% sodium chloride solutions (saline) usually at a concentration of 1 to 3 $\times$ 10^{11} phage per ml. Before use in the metabolism experiments described below, the required amount of saline suspension was diluted to about 10 ml. with tryptose broth and kept at room temperature for at least one hour. As described previously (11) this procedure facilitated dispersal of the virus particles and yielded a larger number of plaque forming units. Estimations of virus concentrations, phage per ml., were carried out using the plaque counting method. In the experiments described here the plating efficiency has not been utilized in calculating the ratios of phage particles per cell and the number of phage particles was calculated directly from the plaque count assay.

General Procedure Utilized in Metabolism Experiments

In carrying out experiments on the breakdown of T2r^+ bacteriophage after infection of the host cells, the general procedure used in the present work was as follows. A quantity of tryptose broth, usually 150 ml., was inoculated with *E. coli* B from an 18 hr. slope to give a concentration of approximately 10^7 cells per ml. The culture was aerated at 37° C. until the concentration of cells

reached 2 to 7 $\times$ 10^8 per ml., the density being estimated spectrophotometrically as previously described (11). After the culture was chilled, it was centrifuged at 4300 *g* at 5° C. in an angle centrifuge to sediment the cells. The cells were then resuspended in tryptose broth, previously warmed to 37° C., to give the required volume containing 2 $\times$ 10^8 cells per ml., and the suspension was placed in a large pyrex tube equipped with a stopcock to facilitate the removal of samples. To this cell suspension was added a predetermined amount of labelled bacteriophage and aeration of the culture was begun immediately at 37° C. At intervals 14.8 ml. aliquots were transferred to chilled tubes graduated at 15 ml. and containing 0.2 ml. of a dialyzed 1% solution of crystalline egg albumin to act as a protein carrier. Two and one-tenth ml. of 40% trichloroacetic acid (TCA) was then added to give a final concentration of 5% TCA and the tubes were kept in an ice bath for at least 15 min. The precipitate was removed by centrifuging at 4300 *g* for 15 min. in an angle centrifuge at 5° C. and the supernatant carefully decanted off. Radioactivity estimations on these supernatants were carried out using a liquid counter, 10 ml. capacity, of the type described by Veall (16), in conjunction with a conventional scaling unit.

The P^{32} present in the TCA supernatant solution was presumed to be released during the metabolism of the labelled bacteriophage in the infected cells and, calculated as a percentage of the amount of P^{32} originally added in the radioactive phage, was used as a measure of breakdown for the infecting virus. In most experiments utilizing this technique sufficient samples were taken to permit the breakdown of phage to be followed from the time of addition of the radioactive phage until lysis of the culture. Most of the experiments described were carried out with seven different preparations of purified radioactive virus 7, 8, 9, 9A, 11, 14, and 15.

Experimental

Breakdown of P^{32} Labelled Bacteriophage with Single Infection of the Host cells

Experiments were carried out with each of seven different preparations of labelled virus to determine the extent of breakdown when cells, at a concentration of 2 $\times$ 10^8 per ml., were infected with phage in the ratio of one phage per five cells. The first sample was taken immediately following the addition of phage after which the culture was mixed continuously with a stream of sterile air; thereafter samples were removed at closely spaced intervals, treated as described above, and the percentage TCA soluble P^{32} was determined for each.

In Fig. 1 the percentage TCA soluble P^{32} is plotted against time in minutes for three experiments carried out on different days with one radioactive virus preparation (No. 8).The last point on Curve 2 represents a sample taken immediately after lysis of the culture. It is observed that the curves are two-step in shape. Since the latent period of this phage is about 22 min. the second rise began shortly after the time when the first release of newly

formed virus would be expected from the infected cells. In several experiments of this type titrations carried out at intervals indicated an increase in phage beginning at about 25 min.

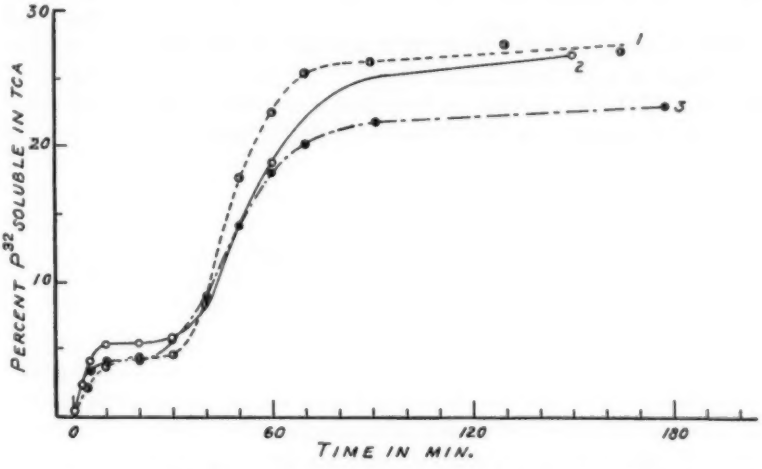

FIG. 1. Release of TCA soluble P^{32} when *E. coli* at 2×10^8 cells per ml. was infected with 0.2 labelled phage per cell. The experiments were performed with one radioactive virus preparation (No. 8). The last point on Curve 2 represents a sample taken after lysis of the culture.

While the height of the first step of the curve indicated 4 to 5% breakdown of the radioactive phage in the first 20 min. or so, the number of counts per minute (c.p.m.) in these samples was only 5 to 10 c.p.m. over the background of the counter (10 c.p.m.). Such a step in the curve was observed consistently in experiments of this type but because of the small increases in radioactivity further experiments were designed to seek more convincing proof of the effect. Consequently, tests were carried out in which the concentration of cells was increased to 6×10^8 per ml., and radioactive phage was added at a ratio of one phage to five cells. Curves of similar shape to those in Fig. 1 were obtained, the first step indicating 3 to 5% breakdown of the radioactive phage. Alteration in the ratio of infection from 0.15 to 0.33 phage per cell, while the cell concentration was retained constant at 6×10^8 per ml., did not alter the height of the first step. These results were interpreted as strong evidence that the first step represented a real breakdown of the phage particles.

Similar results were observed with radioactive virus preparations 7, 9, 9A, 11, 14, and 15. The heights of the first steps in the respective curves represented 3.0, 4.5, 3.5, 4.0, 3.4, 7.0% breakdown and the final amounts of breakdown at lysis of the cultures were 42, 27, 36, 24, 24, 33% respectively. Thus the curves for all seven phage preparations were two-step in shape but considerable variation was observed in the final amount of breakdown undergone by the virus. Time of lysis in these cultures varied between 115 to

310 min. following infection of the cells. There appeared to be no additional release of TCA soluble P^{32} on lysis, since, in several experiments, samples taken some time before lysis and immediately after lysis showed the same percentage breakdown of the virus.

Breakdown of Bacteriophage with Multiple Infection of the Cells

Fig. 2 illustrates the formation of 5% TCA soluble P^{32} in the culture when *E. coli*, at a concentration of 2×10^8 cells per ml., was infected with radioactive phage (Preparation 8) in ratios of 3, 5, and 10 virus per cell. The last

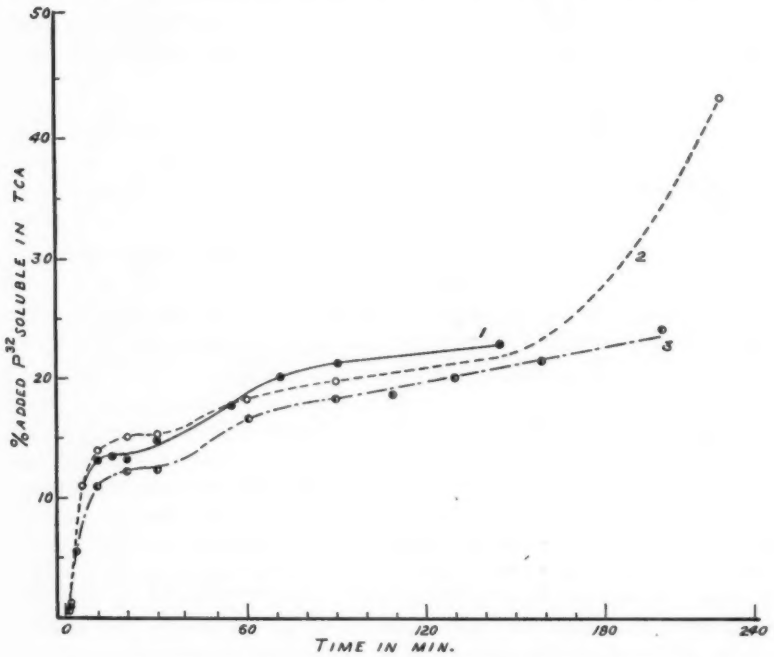

FIG. 2. Relationship between multiplicity of infection with radioactive phage and release of TCA soluble P^{32}. Curves 1, 2, and 3 represent infecting ratios of 3, 10, and 5 phage per cell respectively. Cell concentration was 2×10^8 per ml. in each case.

points on Curves 1 and 3 represent samples taken immediately after lysis of the cells. In a similar set of experiments with Preparation 14A, labelled phage was added to the cells in the ratios of 3, 5, 10, and 15 phage per cell. At 90 min. after infection the breakdown of virus was 21, 21, 25, and 19% respectively. Similar curves were obtained with phage preparations, 7, 9, 9A, and 11 at infecting ratios of three to five phage per cell. For these preparations the TCA soluble P^{32} released at 90 min. after infection was 31, 24, 23, and 24% respectively. Experiments with infecting ratios of 20 to 25 phage per

cell gave variable results. In one instance the breakdown was 25% while two other experiments gave 12 and 13% breakdown. In the latter two experiments lysis of the culture was considerably advanced 30 min. after addition of the phage. In a single experiment in which an attempt was made to follow the breakdown with an infecting ratio of 50 phage per cell the culture lysed completely in less than 20 min. It is probable that this was a case of "lysis from without" as described by Delbrück (5). That the course of the reaction was different from that in the usual multiple infection type of experiment is indicated by the finding that only 2.4% of the P^{32} was present in TCA soluble form in the lysed culture.

Estimations of the amount of phage adsorption were not carried out in most of the experiments of the multiple infection type described above. However, where infectivity titration of the unadsorbed virus was made, the amount of phage adsorbed was 95% or greater 10 min. after addition of phage at ratios up to 10 particles per cell.

While most experiments carried out with multiple infection of cells by radioactive phage conformed to the general picture represented by Fig. 2, certain irregularities were observed from time to time which should be mentioned here. Often there was pronounced flattening of the breakdown curves between 15 to 30 min. following infection of the cells. This was followed by a small increase up to 60 to 70 min. before the curves flattened off and maintained a constant slight slope to the time axis. This effect is well demonstrated in the curves of Fig. 2. However, in a number of experiments of this type the small step between 15 to 30 min. was absent, the curves rising smoothly and flattening off at 20 to 30 min. without any apparent inflection. In many experiments lysis occurred at 150 to 180 min. following multiple infection of the cells. However, there was very great variation in lysis time, some cultures lysing in 90 min. while others took up to 250 min. to lyse. While numerous experiments were carried out to investigate this observation we have been unable to explain the variability. The course of the breakdown reaction with labelled phage did not seem to be influenced by these large variations in lysis time although on isolated occasions, such as represented by Curve 2, Fig. 2, there was a second large rise in the breakdown curve before lysis. This behavior was observed in three experiments for cultures that took about four hours to lyse. In many other similar experiments with lysis periods of four hours this second rise was not observed.

Breakdown of Labelled Bacteriophage When Added at an Interval Following Infection with Nonradioactive Phage

Effect of Variations in the Amount of Normal Phage

When purified nonradioactive bacteriophage ("normal" phage) was added to a cell culture containing 2×10^8 cells per ml. in a ratio of 10 virus particles per cell (primary infection) and, after a five minute interval, radioactive phage was added in the ratio of two phage per cell (secondary infection), the

breakdown of radioactive virus was as shown in Curve 1, Fig. 3. Primary infection with one normal phage per cell gave Curve 2 which is extrapolated to lysis time, while Curve 3 was obtained with a primary infection of one phage

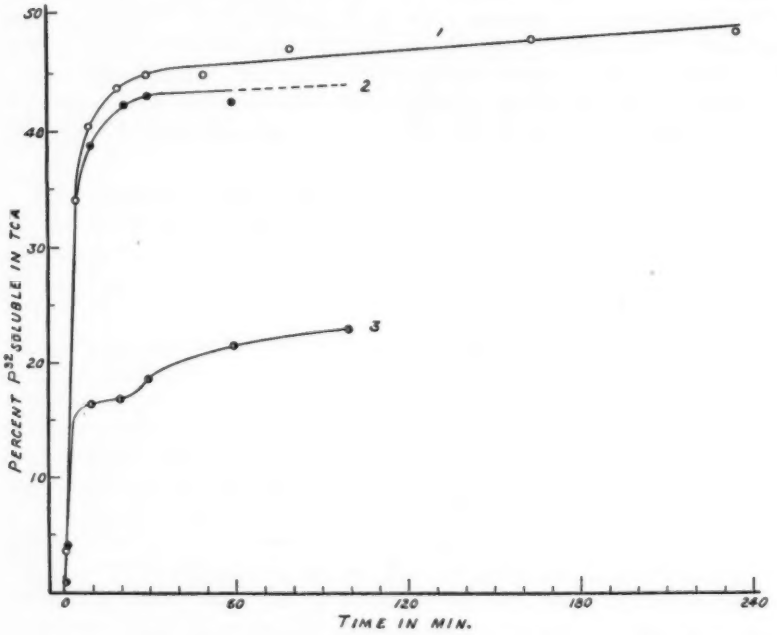

FIG. 3. Effect of variation in primary infecting ratio of nonradioactive phage on breakdown of labelled phage added five minutes later. Primary infecting ratios 10, 1, and 0.15 phage per cell for Curves 1, 2, and 3 respectively at cell concentration of 2×10^8 per ml. The time axis starts with the addition of radioactive phage.

per 6.6 cells. In both these experiments secondary infection was with two radioactive phage (Preparation 8) per cell five minutes after primary infection. In the above mentioned experiment in which one phage per cell was used for primary infection, the actual infecting ratio was probably closer to 1.5 if the plating efficiency of the phage titration is taken into account. Assuming a Poisson distribution some 80% of the cells would be infected. In Fig. 3, the time axis starts at the addition of radioactive phage.

These and other experiments demonstrated that if sufficient normal phage was added to infect most of the cells in a culture, radioactive phage added after an interval of five minutes was broken down rapidly to the extent of 50 to 55%. There was no significant difference when the primary infection was varied between 1 and 10 normal particles per cell. However, when the primary infection was one normal phage per 6.6 cells the percentage breakdown of radioactive virus added after five minutes was very much less. In

most of the experiments of this type the normal phage used for primary infection was taken from suspensions of purified virus. When phage from a freshly lysed culture was used for primary infection a similar picture was obtained. To exclude the possibility that some factor in the lysate, other than phage, might be partially responsible for the extensive breakdown of secondarily adsorbed phage, the following experiment was performed.

More than 99% of the virus was sedimented from a fresh lysate of T2r^+ phage by centrifugation at 120,000 *g* for 60 min. Three ml. of the supernatant solution containing 9×10^7 phage per ml. was added to 150 ml. of a culture of 2×10^8 cells per ml. and after 12 min. three labelled phage per cell were added. The resulting breakdown curve was similar to those obtained with multiple infection of the cells by radioactive virus such as are shown in Fig. 2. This is considered as additional evidence that the increased breakdown of radioactive phage caused by a preliminary infection with normal phage is due to some property of the phage.

Effect of Varying the Interval Between Primary Infection with Normal Phage and Secondary Infection with Labelled Phage

In a series of experiments cell suspensions at a concentration of 2×10^8 cells per ml. were infected with 10 normal phage particles per cell. After an interval two labelled phage (Preparation 8) per cell were added, the interval being varied from 5, 15, 45, and 155 min. from one experiment to another. Within experimental error the curves were the same as Curve 1, Fig. 3. The amounts of breakdown at 60 min. after secondary infection were 46, 41, and 43% for the experiments with 5, 15, and 45 min. intervals. In the experiment with 155 min. between primary and secondary infection lysis occurred 25 min. after the addition of radioactive phage; the amount of breakdown at lysis was 47.5%. When the interval was reduced to one minute the shape of the breakdown curve was similar to Curve 1, Fig. 3, but the total amount of breakdown was much less, amounting to 33% 60 min. after infection.

Effect of Varying the Ratio of Secondary Infection with Radioactive Phage

In a further series of experiments cultures containing 2×10^8 cells per ml. were infected with three normal phage per cell. After an interval of five minutes radioactive phage (Preparation 9) was added at ratios of 0.20, 5, 30, and 60 phage per cell. The shapes of the breakdown curves were similar to that of Curve 1, Fig. 3. Sixty minutes after secondary infection the breakdown was 57, 53, 59, and 58% for the respective infecting ratios. It seems probable from these results that each of the phage particles in the secondary infection was broken down to the increased extent.

Many similar experiments were carried out with other radioactive phage preparations. With primary infecting ratios of 3 to 10 phage per cell, an interval of five minutes, and secondary ratios of 2 to 10 radioactive phage per cell, the curves were invariably similar in shape to Curve 1, Fig. 3. At 60 min. after secondary infection the amount of breakdown of labelled phage

was 56, 46, 59, and 45% for Preparations 7, 8, 9A, and 11. Some preliminary experiments have been performed with T2r^+ phage which was labelled with P^{32} in the synthetic "F" medium described by Cohen and Anderson (4). This purified phage when used to infect cells in broth gave similar results.

As in the experiments on multiple infection described in a previous section, there was a large variation in lysis time in the experiments just described. Lysis usually occurred 160 to 190 min. after primary infection but on occasions occurred as early as 90 and as late as 280 min. There appeared to be no correlation between lysis time and multiplicity of infection of the cells. Considerable variation in lysis time, however, apparently had little influence on the course of the breakdown of infecting phage.

Breakdown of Radioactive Phage When Added at Successive Intervals to a Single Culture of Cells Infected with Normal Phage

Experiments as described above indicated that when cells were multiply infected with radioactive phage on the average about 23% of the P^{32} was broken down to a form soluble in 5% TCA. However when the cells had been previously infected with normal phage the amount of breakdown was increased to about 55%. It was therefore of interest to determine whether several quantities of radioactive phage added at successive intervals to a cell suspension, previously infected with normal phage, would each break down to this increased extent. Such an experiment was carried out as follows.

Normal phage was added to a culture containing 2×10^8 cells per ml. in the ratio of three phage per cell. After five minutes three radioactive phage (Preparation 9) per cell were added and the culture was aerated at 37° C. Equal quantities of labelled virus were added at 23 and 43 min. after addition of the first radioactive phage. Samples were withdrawn from the culture at intervals, and the per cent breakdown determined for each quantity of radioactive phage added; the results are shown in Fig. 4. Each of three quantities of radioactive phage was extensively broken down, the amount of breakdown being about 56, 53, and 74% respectively.

Distribution of TCA Soluble P^{32} Between Cells Infected with Labelled Phage and Supporting Medium

The following experiment was performed to determine whether TCA soluble P^{32} released by the infecting labelled virus was liberated rapidly into the medium or remained in the cell. Information was also obtained on the completeness of extraction of TCA soluble P^{32} by the technique utilized in the metabolism experiments described in preceding sections.

A culture containing 2×10^8 cells per ml. was infected with three normal phage per cell and five minutes later seven radioactive phage (Preparation 11) per cell were added. The culture was aerated at 37° C., and 35 ml. samples were withdrawn 11 and 60 min. after the secondary infection. These were immediately chilled in an ice bath and centrifuged at 5° C. The sedimented cells were resuspended in 35 ml. 5% TCA and allowed to stand in an ice bath

to allow extraction of P^{32} by the TCA. Aliquots were removed after 15 and 75 min. and centrifuged, P^{32} estimations being carried out on the resulting supernatants. Two 14.8 ml. aliquots of the supernatant solution resulting

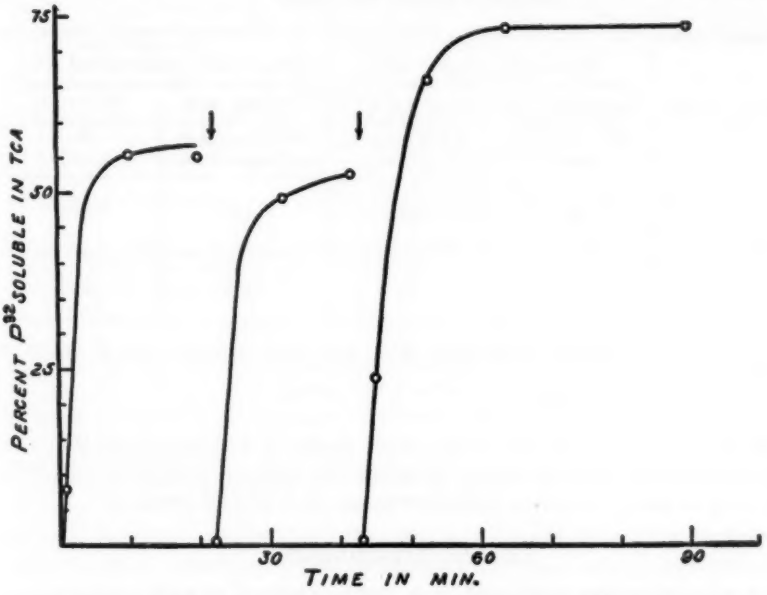

FIG. 4. Breakdown of three quantities of radioactive phage added 5, 27, and 48 min. after infection of 2×10^8 cells per ml. with three nonradioactive phage per cell. Three labelled phage per cell were added in each case. Time axis starts from first addition of radioactive phage and time of other additions represented by small arrows.

from the removal of the infected cells were taken. To the first was added 0.2 ml. albumin and 2.1 ml. 40% TCA in the cold. After this mixture had been allowed to stand 15 min. in an ice bath it was centrifuged and P^{32} was determined in the supernatant. P^{32} was determined in the second aliquot after the addition of 0.2 ml. albumin and 2.1 ml. in water. The results are presented in Table I.

The total TCA soluble P^{32} is obtained by adding the figures in Columns 2 and 4. This amounts to 41% and 47% of the P^{32} added in labelled phage for the 11 and 60 min. samples respectively. It is observed that 12 and 3% of the TCA soluble phosphorus remained in the cells at 11 and 60 min., indicating that the TCA soluble P^{32} released from the infecting phage passed rapidly into the medium. Also, 15 min. treatment with TCA was sufficient to remove whatever soluble P^{32} did remain in the cells. The figures in Column 5 represent TCA soluble P^{32}, P^{32} contained in unadsorbed phage and possibly some breakdown products of intermediate size, while those in Column 4 the corresponding

TABLE I

DISTRIBUTION OF P^{32} SOLUBLE IN 5% TRICHLOROACETIC ACID BETWEEN CELLS AND MEDIUM AT TWO INTERVALS AFTER SECONDARY INFECTION WITH SEVEN RADIOACTIVE VIRUS PARTICLES PER CELL. PRIMARY INFECTION WITH THREE NORMAL VIRUS PER CELL FIVE MINUTES BEFORE ADDITION OF RADIOACTIVE PHAGE*

Time of sample, min.	Sedimented infected cells		Supernatant from infected cells	
	Suspended in 5% TCA 15 min.; P^{32} in extract, c.p.m.**	Suspended in 5% TCA 75 min.; P^{32} in extract, c.p.m.	Treated with 5% TCA; P^{32} in resulting supernatant, c.p.m.	No further treatment; P^{32}, c.p.m.
11	62	59	456	745
60	19	20	569	828

* *Amount of P^{32} added in labelled phage 1257 c.p.m. calculated to same basis as figures in the table.*

** *c.p.m. = counts per minute.*

amounts of TCA soluble P^{32}. From these results it was calculated that the radioactive phage was adsorbed to the cells to the extent of at least 77 and 79% at 11 and 60 min. Another such experiment gave similar results.

Control Experiments

The possibility was envisaged that the breakdown of radioactive phage reported in previous sections might be partially due to noninfective, "dead", phage present in the purified preparations. In view of our earlier work (11) it was felt that the preparations of purified radioactive phage consisted essentially of infective particles. Nevertheless, if only a few per cent of the phage particles were dead, and were broken down completely in the metabolism experiments, the results might be difficult to interpret. It was also possible that a small proportion of dead cells might influence the breakdown of the infecting phage.

Consequently, a number of experiments were performed with dead radioactive phage and dead cells, the percentage breakdown of the phage being measured by the same techniques used in the previous experiments. The cells and phage were killed by heating for 45 min. at 70° C. Thermal inactivation, rather than inactivation with ultraviolet radiation, was chosen since it was felt that this approached more closely the method by which phage and cells would be killed during their routine preparation. The results obtained in these experiments are shown in Table II.

It is observed that in none of these control experiments was there any significant breakdown of dead radioactive phage during the first 60 min. except in the case of dead phage added to a lysate freshly prepared by the action of T2r^+ phage on *E. coli*. Presumably the heat inactivated phage was

TABLE II

CONTROL EXPERIMENTS WITH HEAT KILLED CELLS AND LABELLED BACTERIOPHAGE

Type of experiment	Per cent breakdown of radioactive phage
Dead phage, dead cells	0.6% at zero time, 2.2% at 60 min., 3.8% at 97 min.
Dead phage, live cells	1.0% at zero time, 1.0% at 30 min., 0.8% at 105 min.
Live phage, dead cells	0.4% at zero time, 2.9% at 70 min., 7.8% at 101 min.
Live phage in fresh lysate of T2r^+	0.4% at zero time, 0.9% at 70 min., 5.9% at 142 min.
Dead phage in fresh lysate	0% at zero time, 14% at 28 min., 34.2% at 120 min. 0.2% at zero time, 18.2% at 120 min.
Dead phage added to live cells five minutes after addition of three normal phage per cell	0.5% at zero time, 0.5% at 30 min., 0.9% at 120 min.

digested by the desoxyribonuclease (DNase) reported to be present in fresh lysates (2). It is worth noting that, after 10 min., there was no adsorption of the heat killed bacteriophage to either live or dead cells.

In the experiments on single infection of the cells with labelled phage, evidence has been presented that the initial step in the curve represented a real and significant breakdown of the virus to the extent of about 5%. It still seemed possible, nevertheless, that this breakdown might result from the destruction of a small amount of radioactive pentose- or desoxypentosenucleic acid present in the virus preparation as an impurity, rather than a metabolic rearrangement of the structural constituents of the virus. To gain information on this point experiments were carried out in which purified labelled phage preparations were subjected to the action of ribo- and desoxyribonuclease.*

In a typical experiment the amount of TCA soluble P^{32} in the purified virus was 0.5%. This amount was not increased by incubation with crystalline ribonuclease (RNase) at 37° C. for 30 min., while the TCA soluble P^{32} increased to 1.73% upon incubation with DNase for 30 min. Neither enzyme reduced the infectivity of the phage under the above conditions. Upon heating the phage for 15 min. to 60° C. the infectivity was reduced to 50% and after 60 min. at 70° C. the infectivity was less than 1% of the original. When DNase was allowed to act on these heated preparations 20.8 and 89.5% respectively of the P^{32} was converted to a TCA soluble form. In neither case did RNase liberate any TCA soluble P^{32}. There was thus a rough correlation between the amount of inactivation of the phage and the amount of acid soluble P^{32} formed by action of DNase on it. This finding was consistent with the previous observation that a considerable portion of the P^{32} was converted to an acid soluble form when heat killed labelled phage was aerated in a fresh T2r^+ broth lysate.

* *We are indebted to Dr. G. C. Buller for a specimen of highly purified desoxyribonuclease.*

From these results it appeared probable that the small amount of acid soluble P^{32} formed by the action of DNase on freshly purified phage arose from a small amount of dead phage in these preparations. Next, an effort was made to determine whether labelled phage subjected to the action of DNase would behave in the same manner as an untreated preparation upon infection of the host cells.

Accordingly, a freshly purified preparation of labelled phage was divided into two portions. One of these was treated with highly active DNase for 30 min. at 37° C. This procedure increased the proportion of TCA soluble P^{32} from 0.4 to 1.98%. The treated and untreated phage were then used to infect two cultures of *E. coli* containing 2×10^8 cells per ml. in the ratio of one virus to five cells, and the cultures were aerated at 37° C. Samples were taken at intervals for determination of TCA soluble P^{32}. When allowance was made for the acid soluble P^{32} which had been liberated by the enzyme, the two breakdown curves were the same within experimental error. Thus preliminary treatment with DNase did not influence its breakdown when cells were singly infected with the virus.

Discussion

Previous work by Cohen (3) and Lesley *et al.* (11) with T2r^+ phage and Kozloff and Putnam (9) with T6r^+ phage has indicated that the P^{32} in labelled virus is almost exclusively contained in the desoxypentose nucleic acid moiety and is incorporated into its structure. When the host cell was infected with labelled virus a certain portion of the P^{32} changed to a form soluble in 5% trichloroacetic acid. Since the infecting virus generally contained less than 0.5% of its P^{32} in a TCA soluble form, it seems reasonable to assume that the formation of TCA soluble P^{32} during the course of infection did represent degradation of the virus nucleic acid. Live radioactive phage did not break down to any appreciable extent when added to a fresh lysate. Therefore an extracellular breakdown mechanism, such as the liberation of an autolytic enzyme into the medium, seems to be excluded. The control experiments further indicated that the formation of acid soluble P^{32} resulted largely from the degradation of fully infective virus rather than from the breakdown of a portion of dead phage in the purified preparations.

That the breakdown may be extensive is indicated by the observation of Putnam and Kozloff (14) that on lysis of cells infected with labelled T6r^+ phage about 50% of the TCA soluble P^{32} was inorganic phosphate. Using the formation of TCA soluble P^{32} during infection as a criterion, the breakdown of infecting phage proceeded in different ways depending on the method of infecting the cells.

It would appear that the first 5% step in the single infection curve represents the breakdown of invading phage under the simplest set of conditions. A reasonable explanation of most of our results can be devised if it is then assumed that infection of the cells with phage stimulates the formation of

some mechanism in the infected cell which breaks down phage particles arriving at a later time to the extent of about 55%. For the purpose of discussion this phenomenon will be called the "stimulation effect", and the initial infection with normal phage will be said to "stimulate" the cells. The stimulation is produced rapidly after adsorption of phage. Radioactive phage added one minute after primary infection was broken down by about 33% indicating that the effect was already apparent; the maximum effect was observed within five minutes of primary infection and was probably achieved within two to three minutes. Once cells were stimulated their capacity for breaking down phage remained undiminished until at least 25 min. before lysis; possibly the stimulation remains until immediately before lysis.

It is postulated that the stimulation effect may be a surface phenomenon and that phage adsorbed to the cell before it is produced are inaccessible to later breakdown by it, perhaps owing to their penetration of the cell. A tentative explanation of the multiple infection curve can then be made as follows. When phage is added to cells to give multiple infection, the greater portion is adsorbed in the first two minutes. It is suggested that these phage are broken down by about 5%. In the process the stimulation effect is initiated and phage adsorbed after more than two minutes suffers the full stimulation breakdown of 55%. The curve is thus the resultant of two processes. Let us consider two experiments with a labelled phage preparation in one of which the infecting ratio is 10 and in the other 100 phage per cell, the cell concentration being held constant at 2×10^8 per ml. Under these conditions about 75% of the phage is adsorbed in two minutes in each instance. Assume that this phage is broken down by 5% and the remainder, adsorbed at a later time, is broken down by 55%. In each case it may be calculated that the maximum amount of breakdown is 17.5%. This theoretical computation thus shows that the breakdown curves for widely differing multiplicities of infection should achieve the same maximum value, which, in fact, is close to the determined average value of about 23% breakdown for this type of experiment. The difference between theoretical and experimental values may result from the assumption that stimulation does not begin until two minutes after mixing phage and cells. In practice stimulation breakdown is apparent after one minute and the calculated figure should therefore be a minimum value.

The small plateau often found in the experimental multiple infection curves between 15 and 30 min., and the extensive rise found on isolated occasions before visible clearing of the culture, are probably due to lysis of some cells. Readsorption of the progeny on other infected, and consequently stimulated cells results in release of TCA soluble P^{32} from the isotope contained in the progeny and therefore a rise in the curve.

When cells are singly infected with labelled phage about 5% of the P^{32} appears in an acid soluble form. Whether this is removed from the phage particle after adsorption and before entry into the cell or whether it is released by an intracellular reaction is unknown. It is assumed that a considerable

portion of the remaining P^{32} is transferred to the first generation progeny. At the end of the 21 min. latent period the progeny is released into the medium and rapidly infects the remaining cells with, on the average, about 15 phage per cell under the conditions used in the present experiments. The second step is then a multiple infection breakdown curve of the type discussed above. Assuming that all the first generation phage is broken down by 55% by the stimulation effect during the second cycle of infection, a transfer to the progeny of 50% of the P^{32} in the original labelled phage would be necessary during the single infection cycle to account for the height of the second step in the breakdown during the second cycle.

It has been demonstrated by Putnam and Kozloff (14) that, when cells are multiply infected with labelled T6r^+ phage, 22 to 42% of the P^{32} appears in the progeny. Our own results (unpublished) show a transfer of 15 to 25% P^{32} to progeny in experiments of a similar type with T2r^+ phage. There is, consequently, a considerable discrepancy between the amount of P^{32} transferred to progeny in experiments of the *multiple* infection type, and the amount which should, according to the above explanation, be transferred during *single* infection to account for the second step in the breakdown curve.

This discrepancy may be resolved in either of two ways. Firstly, the explanation given above for the second step may be incorrect and the rise could be accounted for by release of acid solution P^{32} upon lysis of the singly infected cells. It is felt that this is unlikely since, during the large number of experiments carried out in the course of this work, visible clearing of a culture has not been observed to release additional TCA soluble P^{32}. Secondly, the proportion of P^{32} in the infecting phage which is available for incorporation into progeny in the multiple infection type of experiment is reduced by the stimulation breakdown reaction. The stimulation breakdown does not enter the picture during the first cycle of single infection, and it is thus possible that the proportion of P^{32} transferred to progeny in this cycle is a good deal higher than in multiple infection. One possible means of settling this point would be to determine the transfer of P^{32} to progeny for a virus which shows no stimulation effect, and preliminary experiments along these lines have suggested that T^1 or T^7 coliphages may be suitable.

The mechanism of the stimulation effect and of phage breakdown by it are unknown but the process bears some resemblance to fertilization of the ovum by a sperm. Apparently a single sperm penetrates the egg and some mechanism is set in operation which bars the entry of further sperms (12, 15). It may be that infection of the cell by phage induces the rapid formation of a nuclease or protease system at the surface and as more phage are attached to the cell they are digested. Up to the present, this breakdown reaction has been investigated using as an arbitrary measure the formation of TCA soluble P^{32}. However, it seems plausible to assume that acid soluble P^{32} is not the only product of the reaction and that other fragments of phage breakdown which are not soluble in TCA are also present. Investigation of these products may give some clue to the mechanism of the reaction.

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STUDIES ON THE RELATIONSHIP BETWEEN VIRUS AND HOST CELL

III. THE BREAKDOWN OF P^{32} LABELLED T2r^+ BACTERIOPHAGE ADSORBED TO *E. COLI* PREVIOUSLY INFECTED BY OTHER COLIPHAGES OF THE T GROUP[1]

By R. C. French, S. M. Lesley, A. F. Graham, and C. E. van Rooyen

Abstract

Infection of *E. coli* with T2r, T4r^+, T4r, T5, T6r^+, or T6r phages induces the formation of a mechanism which extensively degrades P^{32} labelled T2r^+ phage adsorbed to the cell shortly afterwards, about 50% of the P^{32} being converted to a form soluble in 5% trichloroacetic acid. Each of the above viruses is as efficient in this respect as a preliminary infection with T2r^+ phage itself. Previous infection of the cells with T1, T3, or T7 phages does not stimulate this mechanism to break down labelled T2r^+ virus. When T1, T3, or T7 phage, then T2r^+ phage, and finally P^{32} labelled T2r^+ phage were added to cells, with an interval of several minutes between each addition, the results indicated that adsorption of T2r^+ to the cell was not sufficient *per se* to stimulate the breakdown of labelled phage. Apparently actual infection of the cell by T2r^+ virus was required before the breakdown mechanism was induced.

Introduction

In previous papers (6, 8) it was reported that infection of *E. coli* by T2r^+ bacteriophage labelled with P^{32} resulted in the rapid appearance of acid soluble P^{32} in the culture. This low molecular weight P^{32} was considered to result from a chemical breakdown of the virus nucleic acid during, or shortly after, its adsorption to the host, and was used as a measure of the process.

When the labelled phage was added to a cell culture in an amount sufficient to ensure that, of the cells infected, practically all had adsorbed only one virus particle, the breakdown amounted to about 5%. However, when the cells were first infected with purified nonradioactive T2r^+ phage and this was followed five minutes later with labelled virus, the latter was rapidly broken down by about 55%. These results led to the suggestion that infection of the host cell with T2r^+ phage stimulated the rapid formation of some mechanism in the cell which extensively degraded other T2r^+ particles adsorbed to the infected cell at a later time.

In order to gain more information about this "stimulation effect" it was of interest to determine whether other phages would stimulate the cells to break down T2r^+ phage in a similar manner. Accordingly, experiments were performed in which P^{32} labelled T2r^+ phage was added to cells previously infected with other coliphages of the T group (1), and the extent of the breakdown of the labelled phage was determined. The results are reported in the present paper.

[1] *Manuscript received February 12, 1951.*

Contribution from Connaught Medical Research Laboratories, University of Toronto, Toronto, Ont.

Aided by a grant from the National Cancer Institute of Canada.

Material and Methods

T2r^+ bacteriophage was labelled with P^{32} and purified as described previously (7).

The general method of carrying out the experiments was as follows. Bacto-tryptose broth was lightly inoculated with *E. coli* from an 18 hr. slope and aerated at 37° C. until the cells reached a concentration of 3 to 6 × 10^8 per ml. After sedimenting the cells they were resuspended in the required volume of fresh warm broth at a concentration of 2 × 10^8 cells per ml. The cells were then infected by adding a large excess of one of the T coliphages and after a predetermined time labelled T2r^+ phage was added. Aliquots were removed from the culture at intervals and the amounts of P^{32} soluble in 5% trichloro-acetic acid were determined as described in detail elsewhere (8). The acid soluble P^{32} was calculated as a percentage of the amount of P^{32} added with the labelled virus and thus used as a measure of breakdown of T2r^+ phage.

Experimental

The Breakdown of Labelled T2r^+ Phage Added to Cells Infected with Other T Coliphages

In a series of experiments cultures of *E. coli* were infected with each of the seven coliphages of the T group, and also with the fast lysing *r* (4, 5) mutants of phages T2, T4, and T6 (primary infection). Sufficient excess of virus was added to ensure that practically all the cells would be rapidly infected. After an interval labelled T2r^+ phage was added to the cultures (secondary infection) and a sufficient number of samples were taken to determine the course of the breakdown. The results shown in Table I represent the maximum breakdown for each experiment.

It is observed that the 10 phages used for primary infection fall into two distinct groups, T1, T3, and T7 do not stimulate the cells to break down T2r^+ phage, whilst all the other phages do induce the stimulation effect just as efficiently as a primary infection with T2r^+ phage itself.

Some variation in results was observed when primary infection of the cells was accomplished with T1, the amount of breakdown of secondarily adsorbed T2r^+ being as high as 13.5% and as low as 3.5%. There was generally a partial visible clearing of the culture 15 to 20 min. after infection with T1, corresponding roughly to the end of the latent period of this virus, but lysis of the culture seldom proceeded to completion. It seems possible that a proportion of the cells, varying from one experiment to another, became multiply infected with T2r^+ phage despite the primary infection with T1, and lysis inhibition of these cells as described by Doermann (3) ensued. It was determined that about 96% of the cells were infected five minutes after the addition of T1 virus in the ratio of five virus particles per cell.

While the above experiments showed there was no stimulation of the cells by Tl, T3, or T7 viruses to break down T2r^+, it was considered possible that after infection by one of these viruses, adsorption of T2r^+ phage to the infected

TABLE I

THE BREAKDOWN OF P^{32} LABELLED T2r^+ BACTERIOPHAGE ADSORBED to *E. coli* PREVIOUSLY INFECTED BY OTHER COLIPHAGES OF THE T GROUP

Primary infection		Interval between primary and secondary infection, min.	Infecting ratio of labelled T2r^+ phage to cells	% breakdown of T2r^+ phage
Phage	Infecting ratio of phage to cells			
T1	10	8	3	7.0*
T2r^+	5	5	1	45††
T2r	10	5	1	47**
T3	10	7	1	4*
T4r^+	5	5	1	53††
T4r	5	5	1	52**
T5	5	33	5	46†
	10	15	1	41†
T6r^+	5	5	1	49††
T6r	5	7	1	52**
T7	10	7	1	2.1*

* *Twenty minutes after addition of labelled T2r^+ phage; lysis complete in all except T1 experiment.*

** *Thirty minutes after labelled T2r^+; lysis complete in each case.*

† *Forty minutes after labelled T2r^+; neither culture fully lysed.*

†† *Sixty minutes after labelled T2r^+.*

cells might stimulate them to break down radioactive T2r^+ added shortly afterwards. The following is an example of a number of experiments designed to test this point.

A cell culture was infected by the addition of 10 particles of T1 phage per cell and five minutes later T2r^+ phage was added in the ratio of five particles per cell. After a further three minutes to allow adsorption of this phage, radioactive T2r^+ was added in the ratio of one particle per cell. Aliquots were then withdrawn from the culture at intervals and the percentage breakdown of radioactive phage determined. The results from this experiment are shown in Curve 3, Fig. 1, in which the time axis starts with the addition of labelled phage. A colony count of surviving cells five minutes after addition of T1 phage indicated that 95.5% had been infected with T1. Two minutes after the addition of radioactive T2r^+ phage the overall adsorption of T2r^+ was 97.1%. At this time a titration of infective centers on B/1 cells, a mutant strain of *E. coli* resistant to T1, demonstrated that 15% of the cells were infected with T2r^+. The culture had cleared markedly 10 min. after the addition of radioactive phage.

A repeat experiment with T1 as the primary infecting phage gave results illustrated in Curve 1, Fig. 1. In this experiment there was little or no visible lysis of the culture 32 min. after the addition of labelled T2r^+ phage. The

extended lysis period and increased breakdown indicate that, previous to the addition of labelled phage, T2r^+ infection was established in a much larger proportion of the cells here than in the first experiment.

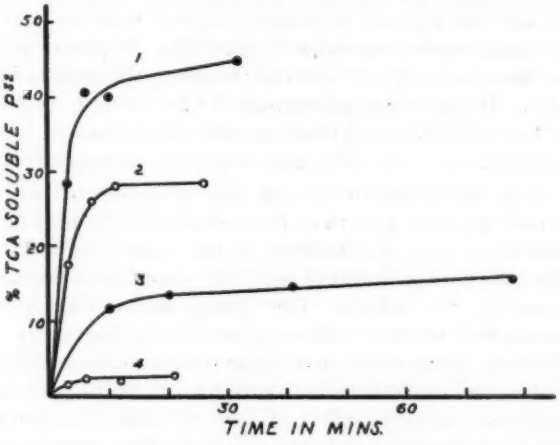

FIG. 1. The breakdown of P^{32} labelled T2r^+ bacteriophage added to previously infected *E. coli*. Curves 1 and 3, primary infection with 10 T1 phage per cell, 5 T2r^+ per cell added after five minutes followed by one radioactive T2r^+ phage per cell after a further interval of three minutes. Curves 2 and 4 obtained under identical conditions except that 10 T3 and 10 T7 particles per cell respectively substituted for primary infection with T1. Time axis starts with the addition of labelled T2r^+ phage. Lysis complete when last samples taken for Curves 2 and 4. No visible lysis of culture at last point on Curve 1, but marked lysis at 10 min. for Curve 4.

Further experiments of the same type were performed in which T3 and T7 phage were substituted for T1 in the primary infection. Curves 2 and 4 show the results obtained in two experiments with T3 and T7 phages respectively. It is interesting to note that for the experiment represented by Curve 2 the lysed culture contained 5.2×10^9 T3 phage per ml. and 1.3×10^9 T2r^+ phage per ml., while the experiment represented by Curve 4 yielded a lysate containing 9.6×10^9 T7 phage per ml. and less than 10^7 T2r^+ phage per ml. Similar results were obtained in other such experiments.

Discussion

The results presented in this paper demonstrate that the stimulation breakdown of labelled T2r^+ phage induced by previous infection of the cells with T2r^+ is also brought about by previous infection with T4r^+, T6r^+, T2r, T4r, T6r, and T5 coliphages. These phages are no less efficient that T2r^+ phage in producing the effect since labelled T2r^+ adsorbed to cells infected by them

is degraded as extensively as when adsorbed to T2r^+ infected cells. Infection with T1, T3, or T7 viruses does not stimulate the cells to break down T2r^+ phage.

When T7 phage, then T2r^+ phage, and finally labelled T2r^+ phage were added to cells, with an interval of several minutes between each addition, the radioactive phage was broken down by only 3%. Apparently the adsorption of T2r^+ to these infected cells did not stimulate the cells to break down the labelled T2r^+. However, the adsorption of T2r^+ phage to cells previously infected with T1 or T3 viruses did result in some breakdown of labelled T2r^+ added shortly afterwards. The following explanation is suggested.

When T2r^+ phage was added to the cells previously infected with T1 or T3 phages it interfered with the growth of these viruses in the manner described by Delbrück and Luria (2). A considerable, and in the case of T1 a variable proportion of the cells became infected with T2r^+ virus, thus becoming stimulated. Consequently, the labelled T2r^+ phage added shortly afterwards which became attached to these cells was extensively degraded. However, T2r^+ phage adsorbed to the surfaces of those infected cells which were sufficiently advanced in their growth cycle to give T1, T3, or T7 progeny on lysis, and thus to resist the interfering effect of T2r^+, did not stimulate the breakdown mechanism. It appears probable therefore, that mere adsorption of T2r^+ phage to the cell surface may not be sufficient to induce the stimulation breakdown effect; the cell must be infected with T2r^+ virus before the effect becomes apparent.

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NOTE

A Note on the Postoperative Increase in Iodine Excretion

In a previous communication to this Journal (2) we described a sudden increase in the urinary excretion of iodine following surgical operations. Later (1) it was shown that the inorganic iodide of the body tissues was not the source of the extra iodine. Although in those studies special pains were taken to exclude the use of iodine preparations pre- and postoperatively, either as medications or antiseptics, no restrictions were imposed as to the type of suture material used by the surgeons.

In a recent review Rapport and Curtis (3) suggested that iodine might be released from gut sutures. Acting on this suggestion we have now investigated this subject by the following means:

(1) Analysis of catgut for its iodine content. This common suture material is apparently frequently dispensed in preservative solutions of various iodine compounds.

(2) Determination of the urinary excretion of iodine in patients subjected to surgical trauma where all suturing was done with silk, no gut being used.

Results

Two separate analyses on a fresh unused sample of catgut showed the gut to have an iodine content of 367 μgm. per 100 mgm. In this particular sample this gave a value of approximately 8.6 μgm. per cm.

In Table I is shown the results of four patients subjected to surgery, the suturing being "all silk". In none of the four was the rise in the iodine

TABLE I

IODINE EXCRETION FOLLOWING SURGICAL OPERATIONS
(NO CATGUT WAS USED IN SUBJECTS 1–4, WHILE ITS USE WAS NOT RESTRICTED IN THE PREVIOUS SERIES)

Subject	Age	Sex	Disorder	Urinary iodine excretion, μgm. per 24 hr.				
				Pre-operative day	Operative day	Post-operative 1	Post-operative 2	Post-operative 3
1	54	M	Umbilical hernia	67	71	44	—	—
2	39	M	Fissure in ano	—	288	222	94	—
3	49	M	Inguinal hernia	—	39	80	85	93
4	66	M	Inguinal hernia	—	218	78	104	138
Average of previous series, 11 cases (2)				180	960	620	475	180

excretion postoperatively comparable with that reported for the previously studied groups (1, 2) where it is likely that catgut sutures were used in the majority of cases.

Discussion

It seems likely from both the iodine content of catgut and the amounts commonly used in surgical procedures that the major part of the previously reported postoperative increase in iodine excretion can be attributed to iodine derived from the sutures.

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Received March 22, 1951.
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CANADIAN JOURNAL OF MEDICAL SCIENCES

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